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REVIEW ARTICLE OPEN ACCESS

Natural Antimicrobial Monoterpenes as Potential Therapeutic Agents in Vaginal Infections: A Review

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Abstract

The recurrence and relapse of vaginal infections in women is a major issue and a challenging pathway to identify and develop new approaches to treatment. In the case of antibiotic therapy, contraceptives, and dietary changes, the recurrence of vaginitis is more common these days. Anaerobic bacteria, *Candida* spp., and trichomonas in the vaginal microflora cause both symptomatic and asymptomatic vaginitis, which includes vaginal inflammation. It changes the vaginal microbiota and decreases *Lactobacilli* spp. growth, which is maintaining the vaginal pH (3.5-4.5) through lactic acid production, antimicrobial peptides, bacteriocin, and bacteriocin-like inhibitory substances. The remarkable antimicrobial activity of plant's producing metabolites like alkaloids, tannins, phenolic compounds, flavonoids and terpenoids for several vaginal infections have been reported in previous studies. Presented review focuses on the pivotal role of monoterpenes, providing a detailed description of thymol, geraniol, limonene, eugenol, eucalyptol, and α-terpineol as antimicrobial molecules in the treatment of vaginal infections. These monoterpenes are very good at killing *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Aspergillus niger*, *Trichomonas vaginalis* and *Candida albicans* which are the main microbes that cause vaginitis. Future research could explore the latent combinations of such monoterpenes as synergistic antimicrobial agents to treat bacterial and fungal vaginal infections, trichomoniasis, among other conditions.

Keywords: Vaginal Infections, Bacterial Vaginosis, Vulvovaginal Candidiasis, Trichomoniasis, Monoterpenes, Antimicrobial Molecule

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INTRODUCTION

Vaginal microbiota contains several healthy microbes that remain in a mutually regulated relationship with the human body.[1] The body produces immunoglobulins (secretory IgA and IgG), mucins, neutrophil gelatinase-associated lipocalin, secretory leucocyte protease inhibitor and β-defensins, as well as other proteins possess antimicrobial action. Collectively, these substances create a strong initial defense against infection.[2] *Lactobacilli* spp. fortifies the defensive mechanistic pathway against the colonization of unhealthy microbes.[3] The vaginal milieu becomes more prone to this kind of invasion because of continuous transformations in the vaginal microbiota during the menstrual cycle, menopause, and hormonal changes during the pregnancy.[4] The DNA sequencing method describes different types of dominant *Lactobacilli* spp. in the vaginal microenvironment. These include four *Lactobacillus* spp. (*L. gasseri*, *L. crispatus, L. iners*, and *L. jensenii*) which are also known as Community State types (Type-I, II, III, and V) along with some anaerobic bacteria linked to bacterial vaginosis (BV) that can cause vaginal infections but remain dormant due to *Lactobacilli* acting as their defense.[5] *Lactobacilli* spp. is associated with the production of L-isomers and D-isomers of lactic acid in the vagina. *Lactobacillus iners* produces L-isomer of lactic acid because it does not constitute the gene coding for hybridization of D-isomer.[6] To regulate MMP-8 (matrix metalloproteinase) production, D-isomer modulates metalloproteinase-inducing activity. The MMP-8 is having the potential of degrading cervical plugs in vagina that prevent bacteria to reach to the upper part of genitals.[7] The microbiota of vagina comprises immune-responsive specialized cells known as natural killer cells, neutrophils, macrophages, cytotoxic cells and helper cells. It also contains specialized receptors known as NLRs and TLRs, which can detect invasive pathogens and initiate the release of interleukins and TNF-α. These immune responses are regulated by the MAPK signaling pathway.[8,9] The development of resistance by microorganisms in several antibiotic therapies sparked the interest of researchers in antimicrobial plant extracts and their secondary metabolites.[10] Traditional studies reported a huge number of antimicrobial plants with their secondary metabolites, which act as antimicrobial molecules against bacteria, fungi, and disease-causing pathogens.[11] Plant secondary metabolites included alkaloids, saponins, monoterpenes, sesquiterpenes, and flavonoids, exhibit remarkable *in-vitro* activity against those pathogens that causes vaginitis.[12]

Vaginitis

Vaginitis reflects the changes in vaginal microbial community that is frequently correlated with several vaginal infections like as candidiasis, trichomoniasis, bacterial vaginosis, cytolytic vaginosis and aerobic vaginitis.[13] Higher vaginal pH is directly associated with increased vulvovaginal infections due to the decrease in lactic acid concentration and the number of *Lactobacilli* spp. It results in a spectrum of symptoms such as irritation, foul odor, burning sensation, frequent urination, vaginal discharge, abdominal pain, and preterm delivery.[14] Certain types of contraceptives, improper hygiene, dietary changes, lifestyle, and antibiotic resistance in the body have caused women to experience recurrent vaginitis episodes.[15] The synchronous occurrence of these infections not only misleads the diagnosis, but it may also increase the probable risk of longer-term persistence of these microbes in the body, which may lead to overlapping of the symptoms and failure to identify an adequate treatment regimen.[16] Major vaginal infections, including VVC vulvovaginal candidiasis, BV bacterial vaginosis, TV trichomoniasis, AV aerobic vaginitis and CV cytolytic vaginosis, with their associated changes in vaginal microflora, common causes, symptoms, and associated risk factors are listed in Table 1.[17,18]

Vulvovaginal Candidiasis (VVC)

Candidiasis is a commonly occuring inflammatory condition caused by *Candida* spp., which majorly affects about 70-75% women in their reproductive age.[19] The clinical symptoms of candidiasis are characterized by itching and redness of the vulva and vagina, a burning sensation, clumpy white discharge, and a frequent urge for urination.[20] Onset of VVC is associated with many of the triggering factors, such as antibiotic resistance (after long term antibiotic therapy), oral contraceptives (which increase

Table 1. Causes, symptoms with associated risk factors of different types of vaginitis

Types of vaginitis	Changes in vaginal microflora	Common causative reasons	Symptoms	Associated risk factors
Vulvovaginal Candidiasis (VVC)	Increased number of *Candida albicans,* higher vaginal pH	Colonization of *Candida albicans*, Antibiotic resistance, Hormonal replacement therapies	Vaginal edema and inflammation with vulvar excoriations	Uncontrolled diabetes, Multiple sex partners, oral contraceptives, douching and smoking
Bacterial Vaginosis (BV)	Decreased number of *Lactobacillus,* higher vaginal pH, excessive growth of anaerobic bacteria	Colonization of *Gardnerella vaginalis, Mycoplasma hominis* and BV associated bacteria	Pelvic inflammation, cervicitis, discharge with foul odour	Frequent use of antibiotics, overlapping infections, multiple sex partners
Trichomoniasis (TV)	Destruction of vaginal and urethral epithelial lining, higher vaginal pH, Decreased number of *Lactobacillus*	Colonization of trophozoite *Trichomonas vaginalis,* promote in growth of anaerobic bacteria	Dysuria, Vulvar pruritis, pelvic inflammation, UTI	Sexually transmitted disease, HIV, smoking and douching
Aerobic Vaginitis (AV)	Thinning of vaginal epithelium, Decreased number of *Lactobacillus*, vaginal pH over 6	Increased number of aerobic bacteria	Green vaginal discharge with foul smell, vaginal mucosal inflammation, and ulcer formation	Lower level of oestrogen,
Cytolytic Vaginosis (CV)	Lysis of vaginal epithelium, Increased number of *Lactobacillus*, vaginal pH lesser than 3.5, excessive H_2O_2	Overgrowth of *Lactobacillus* spp.	White frothy vaginal discharge, dyspareunia, vulvar dysuria and pruritis, vulvodynia	Frequent use of antifungals

estrogen levels), hormonal replacement therapies, tight fitting clothes, multiple sex partners, and uncontrolled diabetes. Gene polymorphism is a predisposing factor for the occurrence of VVC, includes single nucleotide polymorphism (SNP in that gene, which codes for mannose binding lectin), dectin-1 receptor, IL-4, and variants of genes of IL-22 with enzymes of regulatory T cells.[21] It is recently reported that out of 284 women, 78% had the history of one or more VVC previous episode, 65% had three VVC annual episodes, and 35% had four or more recurrence annual episodes.[22]

Etiology and pathophysiology

Candida albicans is a main factor in the development of VVC. This fungus changes shape from a normal 'Y' yeast cell to a mycelial 'H' hyphae organism. This process is known as the dimorphic transition. Normally, its 'Y' form remains in commensalism with the host, while the 'H' form found in tissue's samples collected from women, had VVC. When the 'Y' form does not remain in homeostasis with the host and its tolerance becomes defective for the body, it changes into 'H' form. Furthermore, the 'H' form makes a firmly attached biofilm to the vaginal epithelium. The clinical sign of VVC, vaginal discharge is the result of detached 'H' form, cell debris, particularly inflammatory cells, and vaginal fluid.[23,24] The ability of *Candida albicans* to infect other organisms is mostly linked to the Sap family of proteins (ten genes that code for ten proteins, Sap1-Sap10), which are expressed differently in different hosts. This Sap gene differential expression depends on the number of factors, like vaginal pH, the number of 'Y' forms present in the host, and growth stages and types of *Candida albicans*.[25] The natural defensive mechanism of the vaginal environment responds to eliminate the opportunistic pathogen. Cell activation or the secretion of immune mediators by vaginal epithelial cells triggers inflammation during infection.[26] Inflammasomes are the vaginal intracellular multiprotein complexes of NOD like receptors (complexed with ASC protein and procaspase-1 proenzyme) that translate the encountered signals, cause immune activation, and encase the delivery of β-defensin after pathogenic invasion. It results in production of cytokines or interleukins such as IL-1β, IL-18 and lymphocyte activation (T-1 and T-17), which are traced to anti-*Candida* activity.[27,28]

Bacterial Vaginosis or Vaginosis (BV)

Vaginosis caused by specific bacteria, is one of the prevalent vaginal infections that are affecting about 60% of women at the ages of 16 to 45 years. It leads into an abrupt increase in the colonies of etiological microorganisms such as *Gardnerella vaginalis, Mycoplasma hominis, Megasphaera phylotypes* 1 and 2*, Atopobium vaginae, Peptostreptococcus, Sneathia, Bacteroides* spp*., Prevotella* spp*.* and bacterial vaginosis-associated bacteria also classified as (BVAB1, BVAB2 and BVAB3).[29] BV is commonly associated with several complications, like vaginal discharge with foul odor, pelvic inflammation, and infertility, susceptibility to STDs, cervicitis, miscarriages, preterm labour, postpartum infections, and fetal low birth weight. This inflammatory disease is the result of an imbalance in the vaginal microflora, increased vaginal pH, fewer *Lactobacilli* spp*.*, frequent use of antibiotics, and an excessive number of anaerobic bacteria.[30] Amsel criteria are the standardized method used to evaluate the condition of BV microscopically. This criterion includes four characteristics, *i.e*. pH of vagina greater than 4.5, presence of uniform vaginal discharge (white or grey), presence of clue cells (epithelial cells that layered by bacteria), and whiff's test (characterized by presence of the fishy odor, when discharge added to KOH solution) found positive. The Nugent's score is an evaluating system used to assess the presence of Gram-positive morphotypes in numbers *i.e*. normal (0-3), intermediate (4-6) and (7-10) categorized as containing BV.[31,32] Other methods such as, targeted qPCR assays have been used to spot commonly present bacterial species in the discharge of BV patients using 16S rRNA sequencing. Further taxon-directed PCR assays and DNA hybridization studies have also been reported for sensitive detection of BVAB.[32,33]

Etiology and pathophysiology

An excessive number of anaerobic bacteria replace the normally present *Lactobacilli* spp. in BV, resulting in a reduced level of lactic acid, antimicrobial hydrogen peroxide, and some

organic acids.[34] BV is mostly asymptomatic until the vaginal discharge is found to have a fishy odor that is primarily caused by amine production by anaerobic bacteria. This odor is more common after intercourse (due to the presence of semen) and in menstruation (due to the presence of blood), which results in increased alkalinity of the vaginal flora.[35] In both symptomatic and asymptomatic conditions, BV increased the risk of HIV transmission, developing preterm birth, pelvic inflammatory disease and premature membrane rupture.[36] *Gardnerella vaginalis* is the widely described BV causative agent. It is the only bacteria that express virulence factors and the rest are virulent opportunistic pathogens in BV. It is likely associated with the ability to colonize the urogenital tract and form biofilms. It produces mucin-degrading enzymes, resulting in a decrease in the viscosity of vaginal fluid. Cytolysins are known as virulence factors that produced by *Gardnerella vaginalis* that leads into cell death in epithelial cells of vagina after trigger of protein kinase pathway. These cytolysins act as vaginolysins (pore forming toxins) that lyses RBC and vaginal epithelial cells. The presence of cytolysins increases the nutrient availability of *Gardnerella vaginalis.* It also produces some mucosal degrading factors like prolidase, putrescine, and sialidase, resulting in the exfoliation of vaginal membrane. The peptidases of *Gardnerella vaginalis* release amino acids and peptides in the vaginal environment, which stimulates bacterial growth and provides the nutrient facility for other BVABs.[37,38]

Trichomoniasis (TV)

Trichomonas vaginalis causes Trichomoniasis (TV), a protozoan infection that primarily contributes to most sexually transmitted diseases such as STDs, HIV, vaginitis, and urethritis.[39] Trichomoniasis can be asymptomatic for several months, and it is mainly characterized by yellowish-green diffused malodorous thin vaginal discharge with a pH greater than 4.5 and irritation and itchiness in the vulva. It is majorly associated with many reproductive complications, like dysuria, UTIs, vulvar pruritis, painful intercourse, pelvic pain, underweight babies, preterm birth, infertility, and cervical cancer. TV is a white blood cell type flagellated motile protozoan that can exist in more than one cellular form. It can reside in the lumen of the urogenital tract of a woman for a longer time. It releases some cytotoxic proteins that destroy the vaginal and urethral epithelial linings. This parasite decreases *Lactobacillus* colonization, promotes the growth of anaerobic bacteria, and damages the vaginal epithelium, which predisposes women to other urogenital infections.[40,41]

Etiology and pathophysiology

The trophozoite form of TV has unique patterns of protein phosphorylation and a specific signaling mechanism that only works in certain protozoan cell types and the right conditions.[41] The binding of parasites to the host cell is adhesin mediated through TV lipoglycans (TVLG) (the surface molecule of parasites). In humans, galectin-1 is the receptor for TV. TVLG modulates the macrophages and inflammatory response of epithelial cells in the host body.[42] Studies found 11 adhesion proteins on the surface of parasites that cause a high level of binding to epithelial cells. *Trichomonas vaginalis* phagocytoses epithelial cells, fungi, and bacteria in the human host to get nutrients. TV neutralizes host defense proteins by receptor mediated endocytosis or phagocytosis.[43] Phagocytosis or endocytosis of the vaginal microbiota induces dysbiosis (imbalance in microbial's community) that resulted in other vaginal infections like BV due to activation of BVABs. The major cause of this type of imbalance is continuous use of antibiotics which actively trigger human microbe interaction. TV remains in symbiosis with *Mycoplasma hominis* and causes TV-*Mycoplasma* infections. These types of co-infections consume large amount of arginine that leads to cause reduction in nitric oxide produced by natural macrophages in vagina that ultimately interfere with defensive mechanism in human body.[44,45]

Aerobic Vaginitis (AV)

Aerobic vaginitis is also called vaginal dysbiosis or desquamative inflammatory vaginitis and results from an increased number of bacteria such as *Streptococcus* (Group-B)*, Streptococcus viridans, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella* spp*., Enterococcus faecalis, Pseudomonas* spp*., and Citrobacter* spp. and remains asymptomatic in

around 10-30% of the cases.[46] AV is associated with a reduced number or absence of *Lactobacilli* spp. in the vaginal microflora. Symptoms associated with AV included yellowish green putrid vaginal discharge with a foul smell. AV gives a negative Whiff test. Vaginal pH increased over 6, causing burning and inflammation in the vaginal mucosa with severe ulceration. AV is diagnosed by the increased number of parabasal cells, increased interleukins (IL-8, IL-6, IL-1β), and numerous leucocytes, which is most likely the condition in cervical neoplasias in women.[47]

Etiology and pathophysiology

In AV, different types of pathogenic mechanisms are involved that trigger the release of inflammatory cytokines, *i.e.*, interleukins and inhibitory factors (about three folds increase in comparison to BV).[48] Interleukins (IL-6 and IL-8) are linked with an increase in prostaglandins and their delivery. Interleukins activate the leucocytes, which results into thinning of vaginal mucosa and increases the desquamation of epithelial cells. Kallikrein relative peptidases increased in AV resulted in desquamation. Sialidases are hydrolytic enzymes that increase in AV and degrade IgA (the host's defensive molecule). Another reason for AV or DIV is the lower level of oestrogen that might induce the development of DIV in lactating women and in menopause.[49-51]

Cytolytic Vaginosis (CV)

Cytolytic vaginosis or Doderlein's cytolysis or lactobacillus overgrowth syndrome is diagnosed by the presence of an increased number of *Lactobacillus* spp. that resulted in vaginal epithelial cell lysis.[52] It is characterized by abundant white and frothy vaginal discharge, dyspareunia, vulvar dysuria, and pruritis. These symptoms are mostly occured in the luteal phase and during pregnancy. CV is also associated with the presence of false clue cells, in which increased *Lactobacilli* covered the vaginal epithelial cells. More likely CV has symptoms like BV except the pH of vagina (lower than 3.5), absence of *Candida, Gardnerella, and Trichomonas* and Whiff's test results.[53]

Etiology and pathophysiology

Hormones play a vital role in vaginal infections, including CV development. Due to lower pH and elevated glycogen degradation by an increased number of *Lactobacillus* spp. cytolysis in CV occurs. *Lactobacilli* produce isomers of lactic acid. An imbalance in the ratio of these two isomers leads to CV development causing an alteration in extracellular matrix (loproteinase) expression.[54] Patients who have previously received any antifungal treatment often experience CV mimicking the symptoms of VVC. CV is commonly associated with localized vulvodynia. Exposure of the vestibule to an increased number of *Lactobacilli*, low pH, and excessive amount of H_2O_2 often leads to vulvodynia. Researchers have reported over-acidification, an elevated level of l-lactic acid, and numerous fragmented bare nuclei with cytoplasm debris in a wet smear of the affected woman.[55,56]

Monoterpenes: naturally present antimicrobial compounds

Terpene compounds ($C_{10}H_6$) are also classified as isoprenoid's units and their derivatives. When it contains an excessive oxygen element, then it termed as terpenoids. They can exist as monoterpenoids, diterpenoids, triterpenoids, and sesquiterpenoids in nature. The antibacterial, anti-trichomonal and antifungal activity of tea tree oil (TTO) against *Candida*, *Gardenella,* and *Trichomonas* spp. have been widely reported due to the presence of monoterpenoids such as eucalyptol and terpinen-4-ol.[57] Thymol and carvacrol are the main extracted monoterpenoids from *Thymus* spp. that have been reported to have interesting anti-*Candida* activity in VVC.[58] Plants and their associated secondary metabolites such as geraniol, linalool, limonene, and menthol widely reported to possess remarkable antimicrobial activity against a broad spectrum of microbes.[59,60] In a murine model of VVC, nanoemulsions containing eucalyptol and limonene were used. The developed nanoemulsion showed superior anti-*Candida* activity than miconazole cream. In a further study, 16 essential oils were tested against 21 fungi isolates, of which the major monoterpenes citronellol, geraniol, thymol and carvacrol were the monoterpenoids that have been reported for their antifungal effect against all fungi isolates.[61] Monoterpenes such as eugenol, carvacrol, thymol, p-cymene, α-pinene, terpinolene, eucalyptol, and α-terpineol against *Gardenella* spp. have

shown therapeutic success up to 80% for BV.[62] Monoterpenes are the phenolic compounds of plant's essential oils. Phenolic monoterpenes contributed to intrinsic antimicrobial properties in vaginitis. Several monoterpenes and their role in the management of vaginitis with their mechanism of action in treatment of vaginal infections are represented in Figure.[63,64]

Thymol

Thymol or 2-isopropyl-5-methylphenol or hydroxyl cymene is a monoterpene phenolic compound and an isomer with carvacrol, majorly present in extracted oils obtained from plants that belong to families *Apiaceae, Scrophulariaceae, Lamiaceae, Ranunculaceae,* and *Verbenaceae.*[65] Thymol (12-65%) is the major constituent obtained from the plant Thyme (*Thymus vulgaris* and other *Thymus* spp. of the *Lamiaceae* family*)* and many other plants. Thymol has been reported for different pharmacological activities like antiseptic, anti-inflammatory, antinociceptive, antioxidant, local anaesthetic, and most commonly for possessing antifungal and antibacterial properties.[66-70]

Mechanism of action of thymol

Thymol has good antimicrobial action. The antibacterial action of thymol has been evaluated in various *in-vitro* studies that included *Salmonella typhimurium, E. coli, Yersinia enterocolitica, Pseudomonas fluorescens, Proteus mirabilis, E. coli, Sarcina flava, Listeria innocua, Staphylococcus aureus, Micrococcus* spp., and *Bacillus licheniformis.*[71,72] Its antibacterial activity mainly results from changes in the bacterial lipid bilayer and interactions with bacterial DNA. At very low concentrations, thymol can alter fatty acids composition (specially branched 14-methylhexadecanoic acid and 12-methyltetradecanoic acid) of lipid membrane in bacteria and at high concentration it can alter cell membrane integrity and reduces the viability of bacterial cells. The interaction with bacterial genomic DNA included the formation of a junction between bacterial DNA (minor groove) and thymol, which causes destabilization of secondary structures in DNA and results in DNA disaggregation.[73] Another study revealed that in bacterial cell membrane thymol causes uncontrolled intracellular material release, resulting in disturbances in the metabolic actions of bacteria. Thymol is also associated with a reduction in glutathione and nitric oxide production in bacteria.[74] In a microbial cell model, thymol distended DPPS (dipalmitoylphosphotidylserine) monolayers resulted in decreased surface elasticity and a change in the rheological properties of

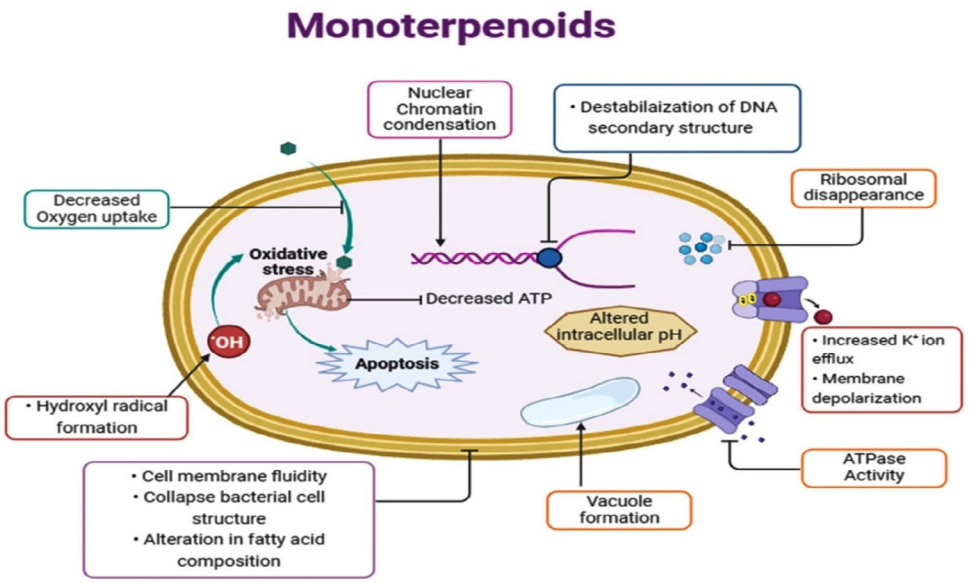

Figure. Schematic representation of mechanism of action of monoterpenes against causative agents in vaginitis

bacterial membrane films. Through this alteration in the cell membrane, thymol can easily permeate into the membrane, resulting in changes in membrane morphology, membrane structural order, membrane fluidity and lipid loading density.[75,76] An antifungal activity study of thymol against *Candida* spp. concluded that it inhibits the ergosterol synthesis in fungal cell membrane and leads into cell impairment. It acts as a chemo sensitizing agent with interfering agents of cell wall. After penetration it reaches to fungal DNA and interferes with DNA synthesis. Thymol also disrupts the oxidative stress response system in fungi when used in combination therapy.[77] A further study reported that thymol inhibits biofilm formation in *Candida albicans* by preventing further biofilm development or adherence. The morphological characteristics of thymol-treated biofilms change due to cell wall shrinkage and leakage in the intracellular matrix In *Candida* biofilms; thymol also inhibits sessile cells.[78] Thymol has superior anti-trichomonia activity.[79] The mode of action against *Trichomonas* spp. includes cell wall lysis and changes in the permeability. Thymol causes apoptosis in *Trichomonas* spp. cells and alters protozoan mitochondrial membrane permeability. It is also reported to interfere with *Trichomonas* metabolism by inhibiting enzyme dihydrofolate reductase, which leads to cell death.[80,81]

Antimicrobial activity of thymol

A study in 1994 reported noteworthy activity of thymol against wide range of bacteria in an effective range, with the lowest minimum inhibitory concentration of 175 µg/mL for three different strains of *Staphylococcus* spp. and 125-245 µg/mL for *E. coli.*[82] Sasso *et al.* identified the antibacterial activity of thymol on *S. aureus* and *E. coli*. In that study it was reported that the ½ MIC shows the highest inhibition for *E. coli* and 1/16 for *Staphylococcus aureus* on vaginal epithelial cells. For thymol, efficient inhibition for adhesion was reported between ½ and 1/32 MIC values.[83] Thymol containing vaginal cream has been reported to show a better response to vaginal and vulvar edema in comparison to the metronidazole-containing vaginal gel.[84] Antifungal activity of thymol in vaginal infections has been reported with highest MIC ≤0.0038 % and 0.0078, <0.015%, v/v for three strains of *Candida* spp. Thymol effectively decreased the number of *Candida albicans* and its biofilm production in the dose range of 64-120 µg/mL. These studies concluded that thymol could suppress the transformation of *Candida* from its yeast to hyphae form.[85] In 2018, a study on five essential oils, including thymol, with seven *E. coli* spp. and twelve *Candida* spp., resulted in promising antibacterial and antimycotic activity with lower MIC values.[86] Cordoba *et al*., evaluated antifungal activity of thymol against four *Candida* spp., including other essential oils and concluded that thymol is an active essential oil at MIC values of 0.8-0.16 mg/L.[87] De Castro et al., reported antifungal activity of thymol with MICs range of 39-78 µg/mL for three strains of Candida spp. Researchers also reported synergistic antimicrobial activity with fluconazole and amphotericin B.[88] Thymol-loaded nanoparticles and nystatin combination have been found to have significant anti-*Candida* activity in comparison to the nystatin group with the best MIC of 0.158 µg/mL.[89] Metronidazole was not as effective against *Trichomonas vaginalis* as thymol, eugenol, and carvacrol extracts. Their hexane extract and methanol extract were more effective.[90] Researchers investigated the anti-trichomonal activity of pure thymol and two nanopreparations, namely nanoliposomes and nanoparticles of thymol, and compared them with metronidazole. The developed formulation resulted in the best activity with a lower inhibitory effect.[91]

Eugenol

Eugenol or 4-Allyl-2-Methoxyphenol is a hydroxyphenyl propene found in volatile oils extracted from a variety of plants, such as *Eugenia cariophylata, Myristica fragrans,* and *Ocimum basilicum,* of the family *Myristicaceae, Lauraceae, Lamiaceae,* and *Myrtaceae*.[92] It is a mainly present in clove oil (45-90%), obtained from *Syzygium aromaticum, Myrtaceae* and widely used for flavouring in the food and cosmetic industry. Traditional medicine uses it for its many pharmacological activities such as antibacterial, anti-inflammatory, antifungal, anticancer, and antioxidant.[93] It has antibacterial, antifungal, and antitrichomonal properties, either alone or in combination.

Mechanism of action of eugenol

A free hydroxyl group present in eugenol is mainly responsible for its antimicrobial activity. The hydroxyl group is attached to bacterial cell proteins and prevents enzymatic action. In Gram-negative bacteria, eugenol penetrates the lipopolysaccharide of cell membrane and causes an alteration in cell structure.[94,95] Eugenol damages the bacterial cytoplasmic membrane, causing potassium ions to leak out and a significant loss of cellular substances, ultimately leading to cell death.[96] Eugenol mainly changes the portrayal of principal fatty acids and increases fatty acids with a low molecular weight.[97] In bacterial cells, eugenol causes cell cytotoxicity by creating reactive oxygen species (ROS). These ROS stop cell growth, damage cell membranes, and damage bacterial DNA, which ultimately causes cell death.[98] It also exhibits noticeable activity against bacterial enzymes like histidine carboxylase, protease, amylase, ATPase.[99] Eugenol causes diminish in production of pyocyanin, violacein elastase (some virulence factors in *Pseudomonas aeruginosa*) and biofilm formation. It also inhibits biofilm production by 65% the gene expression level; especially the QS synthase genes.[100] Eugenol changes the shape of the envelopes of bacteria and fungi due to presence of free hydroxyl group. It is highly effective in reducing colonies of *Candida albicans* and increasing the amount of damaged cells. *Candida albicans* envelope modification is relative to its pathogenicity, so it can cause compromises in host cell adhesion and some morphological transitions.[101] Eugenol affects ion transportation in fungi. Eugenol reduces H^+-ATPase activity in *Candida albicans* and inhibits H^+ extrusion, leading to glucose stimulation. There is a high level of superoxide dismutase in *Candida albicans* that can cause an oxidative stress response. This leads to lipid peroxidation in the cytoplasmic membrane and then cell death.[102,103] Antitrichomonas activity was reported for eugenol in many of the recent studies.[104,105] The mechanism of action against *Trichomonas vaginalis* includes cell wall lysis and cell membrane permeability alteration. Metabolism interference is also one of the main causes for the cell disruption in *Trichomonas* spp.[106]

Antimicrobial activity of eugenol

Eyambe *et al.* determined the antimicrobial activity of eugenol against a number of bacteria such as *Staphylococcus aureus*. The MBC value indicated that it could kill about 99% of bacteria at a minimum concentration. The MIC value was at 115 µg/mL and MBC value was found out at 230 µg/mL.[107] The activity of eugenol against *Staphylococcus aureus*, *E. coli*, and *Candida* spp. is well documented. The study estimated the MIC value of eugenol against *E. coli* to be 1.6 mg/L, for *Staphylococcus aureus* to be 128 mg/mL, and for *Candida* spp. to be 0.88 mg/mL. The study concluded that eugenol exhibited remarkable antimicrobial activity against the causative agents of vaginitis.[108] *Saugella probiogel*, which mainly contained eugenol as an active ingredient, has been tested on a group of 209 women who suffered from BV, VVC, and RVVC. Researchers discovered that eugenol interferes with the biofilms, leading to lower the number of bacterial and fungal pathogens. This resulted in a normal state for 80% of BV cases, 62.5% of VVC cases, and 100% of RVVC cases.[109] Banks and Taghread determined the antimicrobial activity of eugenol and extract of cloves against *E. coli*, *Staphylococcus aureus* (that were resistant to cefotaxime) and *Candida* spp. by disc diffusion sensitive method. It was found that both eugenol and non-polar clove extracts showed a significant inhibition of *Staphylococcus aureus, E. coli,* and *Candida* spp. with $p < 0.001$.[110] The antitrichomonal activity of eugenol showed that the extracts containing eugenol as an active compound cause growth inhibition of *Trichomonas vaginalis* with MIC range of 0.156 mg/mL.[111] Yassin *et al*., used three strains of *Candida* spp. and tested with ethyl acetate extract of clove containing eugenol 60% as an active ingredient. In the disc diffusion method, the zones of inhibition were 14.9, 20.9 and 30.7 for *Candida glabrata, Candida albicans* and *Candida tropicalis*.[112] In a study of 2022, it was found out that eugenol shows better antimicrobial effect than cinnamaldehyde against 18 *Candida* strains that was derived from vaginal strains cultures of infected women. MIC value for eugenol was 455.42 mg/L and MFC value was 690.09 mg/L and all the viable fungal cells were lost within 1 hour.[113] In 2023, another study reported the anti-trichomonal

vaginalis activity of eugenol. In this study the IC_{50} value for eugenol was 1.21 mg/mL which was better than metronidazole.[114]

Eucalyptol (1, 8-Cineole)

Eucalyptol or (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane) is a naturally produced monoterpene majorly obtained (70%) from leaves of *Eucalyptus globulus* as eucalyptus oil and other *Eucalyptus* spp. of the family *Myrtaceae.*[115] Eucalyptol is widely reported to be used in the pharmaceutical, cosmetic and chemical industries. Its pharmacological activities are reported for anti-inflammatory, antiseptic, and antioxidant effects as well as having a strong antimicrobial activity in a broad spectrum.[116] Numerous studies have documented its antimicrobial and antiprotozoal activity in vaginitis.

Mechanism of action of eucalyptol

Eucalyptol was reported to interfere with signaling in quorum sensing in bacteria. Quorum sensing is the technique of communication through chemical signals in microorganisms for promoting their growth, spread, and invasion. Biofilm formation is the result of quorum sensing, which protects the microbes from their external environment. Eucalyptol blocks those receptors that receive signals through different autoinducers.[117] Eucalyptol changes the size and shapes of bacteria cells. In bacterial cells, it causes apoptosis in *Staphylococcus aureus*; when treated, it causes a nuclear chromatin condensation with necrosis in *E. coli* results into a reduction of nuclear chromatin and nucleoplasm.[118] Eucalyptol makes Gram-negative bacteria more likely to have their cell walls damaged, which is a clinical benefit that can cover a wide range of antibacterial activity. Its activity is due to its hydrophobicity, which can synergistically increase the antimicrobial action of other agents.[119] Eucalyptol affects cell membrane permeability, and it enhances the fluidity of the membrane, which results in membrane protein topology and lipid peroxidation. This inhibits bacterial cell respiration. It also increases the oxidative stress and ROS (reactive oxygen species) that inhibit the biological processes of bacterial cells.[120] Eucalyptol was reported to alter membrane fluidity and cell wall permeability of *Candida albicans* by modifying the properties and functions of membrane. Eucalyptol also caused distortion in the fungal hyphae wall, followed by cell membrane disruption. Eucalyptol and other agents can interfere with the activity of mitochondrial dehydrogenase, which is ultimately involved in fungal ATP biosynthesis. The effectiveness of eucalyptol on the H^+-ATP_{ase} of fungal membrane interferes with intracellular pH and fungal cell nutrient uptake. It finally leads to the acidification of intracellular material and cell death. Eucalyptol acts as a potent antifungal for *Candida albicans* and biofilms due to metabolic interference in comparison to fluconazole.[121,122] Eucalyptol hydrophobicity and lipophilic nature allow it to enter the cell membrane of *Trichomonas vaginalis*. It increases cytoplasmic material permeability, which leads to impaired function in Trichomonas. Eucalyptol can interfere with the lipid composition leading to protein denaturation, cytoplasmic substance leakage, membrane disintegration, and cell death. Enzymatic inhibition and immune modulation are other modes of action of eucalyptol in protists.[123]

Antimicrobial activity of eucalyptol

Trinh *et al*. found that eucalyptol and α-terpineol are effective at killing two vaginal bacteria: *Candida albicans* and *Gardnerella vaginalis*. The combination of eucalyptol and α-terpineol potently inhibits the growth of these microorganisms at MIC values (0.125 and 0.065% v/v) respectively.[124] In a study, eucalyptol lowered the number of *Trichomonas vaginalis* in a culture media at the effective concentration of 12.54 mg/mL after 48 hours.[125] Rosemary oil containing eucalyptol showed potent activity against *Pseudomonas aeruginosa* and *E. coli,* with MIC 50 mg/mL and 25 mg/mL, respectively. The antifungal activity for *Candida* reported in this same study, with MIC/MFC value of 1.26 mg/mL.[126] Bogavac *et al*., evaluated the antibacterial and antifungal activity of eucalyptus oil against two strains of of *E. coli*, *Staphylococcus aureus* and *Candida albicans.* The MIC values were reported to be 12.5-25*,* 6.255 and 6.254 µL/mL for *E. coli, Staphylococcus aureus, Candida albicans,* respectively. The study concluded that eucalyptol can be a possible potent antimicrobial agent in vaginal infections.[127] Zhou *et al*., demonstrated that eucalyptus oil containing eucalyptol as major

active constituent having antimicrobial activity against *Candida albicans, E. coli* and *Pseudomonas aeruginosa* with MICs range from 5.5-40.2 µL/mL.[128] In a study of 2021, Sang-Youn *et al*., reported that eucalyptol possessed good antimicrobial and antioxidant effect against *Candida albicans* at MIC value 1.25 mg/mL.[129] In a recent study of 2020, it was demonstrated that eucalyptol is having an efficient anti-trichomonal activity against *Trichomonas vaginalis* at the concentration of 21 mg in a culture media that was found out to be similar as metronidazole.[130] In a further study of 2021, eucalyptol was tested against Gram-positive bacteria, methicillin resistant strains, Gram-negative bacteria, vancomycin resistant strains and *Candida* spp. It showed the inhibition in growth of these microbes in a very prominent range of MIC (0.25-8% v/v).[131]

R-Limonene

Limonene or 1-methyl-4-1-methyl phenyl cyclohexene is a naturally occurring monoterpene (R-Limonene or S-Limonene) containing two isoprene units with a lemon-like odour and is mainly found in citrus oil extracted from lemon, orange, and mandarin peels and in lemongrass oil, *i.e*., extracted from plants of the family Rutaceae.[132] It is reported to possess different pharmacological activities like as antimicrobial, antioxidant, anticarcinogenic, antidiabetic and chemopreventive.[133-135] Limonene has been prominently reported to have strong antimicrobial activities in vaginal infections.

Mechanism of action of R-limonene

The antimicrobial activity and mechanism of action of R-limonene have been well documented. Limonene damages lipopolysaccharide, which is associated with barrel proteins. It also changes membrane permeability and inactivates the bacterial cell envelope.[136] At an optimum concentration, it was found that it leads to the formation of hydroxyl radicals (Fenton-mediated), which result in the oxidation of bacterial DNA and alter the membrane permeability.[137] Collapse in bacterial cell structure (Gram-positive and Gram-negative) was seen when treated with limonene-containing nanoemulsion, which further lead to cell lysis, cell deformation, exudation of cellular material and cell death.[138] The efficacy of antibacterial agents might differ for bacterial cells (Gram-positive and Gram-negative) because of differences in their targeted sites. Limonene also showed difference in its activity for both as Gram-negative bacterial cells are having efficient membrane homeostasis. Limonene is hydrophobic and Gram-negative bacterial cells are prone to develop a hydrophilic barrier to protect themselves, due to which these cells are lightly sensitive for limonene.[139,140] The antifungal activity of limonene included the overexpression of different genes that are associated with cell signaling pathway. It was observed that *Candida* cell deteriorated after treatment with limonene due to altered cell structure and accumulation of reactive oxygen species or ROS that ultimately leads to cell destruction.[141] About 80% of the changes in membrane permeability observed in *Candida* isolates resulted from rupture of cell wall. Researchers found that limonene interferes with pectin methyl esterase and cellulase in fungus cells.[141] Inhibition of biofilm formation by limonene is also reported in various studies against several microbes. Limonene was reported to have better anti-trichomonal activity by causing changes in the morphology of protists. After exposure to limonene, it was observed that it might cause ribosomal disappearance, organelle disintegration, and formation of vacuoles, endoplasmic reticulum dilation, and leakage in cytoplasmic material. It also causes damage to the cytoplasmic membrane and appearance of cytoplasmic vacuoles. Limonene developed holes and projections in cytoplasmic membrane, which finally caused cell death of *Trichomonas vaginalis.* The attachment capacity of protist was also interfered by the presence of limonene.[142,143]

Antimicrobial activity of R-limonene

Vuuren and Viljoen assessed the antibacterial and antifungal efficiency of limonene and eucalyptol in a 1:1 ratio against wide range of bacteria and a fungus isolate. It was resulted into the MIC value range of 2-27 mg/mL for limonene enantiomers for *Pseudomonas* spp*., Staphylococcus aureus* and *Cryptococcus neoformans*.[144] Bassole *et al*., estimated that limonene and its epoxides possessed better antibacterial and antifungal activity against vaginal isolates such as *E. coli, Pseudomonas* spp*.,*

Staphylococcus aureus, and *Candida albicans,* with MIC range of 2.4-3.2%.[145] Essential oil containing lemongrass oil (active constituent limonene) shows a remarkable inhibitory effect against *Streptococcus* spp. with a mean value of 10.07 of the zone of inhibition after 48 hours. Limonene was found to be the second most active antibacterial against *Streptococcus* spp.[146,147] Zahi *et al*. determined the antimicrobial activity of limonene loaded in nanoemulsion (organogel-based) form against *Staphylococcus aureus, Pseudomonas* spp*., and Candida albicans* with a MIC range of 3.14-12.56 µg/mL. The microbial cell's constituent release increased about two folds after the application of limonene organogel.[138] Gundel *et al*. estimated *in vitro* antifungal effect of limonene and eucalyptol in a murine model of vulvovaginal candidiasis. Nanoemulsion containing limonene and eucalyptol showed better antifungal activity in comparison to miconazole cream with $p < 0.05$. The nanoemulsion showed better results due to the smaller size of the globules and larger surface area.[61,148] Another study of 2005 tested the activity (*in vitro* and *in vivo)* of limonene against different specimens of *Candida* spp. on vulvovaginal candidiasis model. It decreased cell viability due to treatment with limonene at EC 50% (444 ± 35 µM) after 8 hr. *In vivo* result was showing significant decrease in number of fungal cells at the concentration of 500 µM of limonene.[139] In another study, an anti-trichomonal activity was examined by using tea tree oil. It was found out that due to presence of limonene the number of protozoan *Trichomonas vaginalis* decreased at IC_{50} 0.06 µL/mL.[149] Nanoemulsion containing limonene with resveratrol showed prominent inhibition of *Staphylococcus aureus* due to the increased skin permeation. Limonene showed synergistic antibacterial activity.[150]

Alpha-Terpineol (α-Terpineol)

α-terpineol or 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol is one of the isomers of terpineols (α, β, γ, and δ) naturally present as monoterpenoid alcohol in essential oils, mainly obtained from *Mentha* spp., *Origanum* spp., *Artemisis* spp., *Thymus* spp., *Pinus* spp., *Salvia* spp., *Melaleuca* spp., and *Narcissus* spp. in high fractions and in small fractions from many more plants. It is characterized by the floral fragrance and flavour.[151] It has been widely known and documented to possess several biological properties as being anticarcinogenic, antioxidant, antimicrobial, anti-inflammatory, antiulcer, skin penetration enhancer, and anticonvulsant.[152,153]

Mechanism of action of α-terpineol

Monoterpenes have been reported to cause hydrocarbon partitioning in microbial cell membranes and disrupt vital functions. It causes lysis of bacterial cell membrane and resulted in to extrusion of cytoplasmic matter with associated ions and respiration inhibition.[154] It disrupts microbial potassium homeostasis, eliminates propidium iodide, forms mesosomes, and modifies the glucose-dependent respiration mechanism in *Staphylococcus aureus* and *Pseudomonas* spp.[155] It also changes the structural functions of the bacterial cellular membrane. Alpha-terpineol resulted to cell shape irregularities, formation of edges in the bacterial nuclear part, condensation of cytoplasm, plasmolysis, and formation of vacuoles in *E. coli* bacterial cells.[156] In *Candida* spp. and *Saccharomyces* spp*.,* it alters membrane permeability and inhibits cell respiration. It also affects the mitochondrial membrane of fungi by inhibiting medium acidification, which in turn interferes with the energy production of the fungi cells. Alpha-terpineol inhibits the germ tube formation; thereby obstructing the conversion of fungi into their mycelial form.[157] Treatment with α-terpineol also causes abnormal hyphae formation due to disruption in the fungi cell wall. It down regulates fungi metabolic pathway and disrupts energy formation.[158] Monoterpene extract contained α-terpineol, which is reported to block acetylcholinesterase in *Trichomonas vaginalis.* Due to acetylcholinesterase blockage, the level of acetylcholine increases, which increases the activation of nicotinic acetylcholine receptors, ultimately leading to muscular contraction and membrane depolarization.[159]

Antimicrobial activity of Alpha-terpineol

Alpha-terpineol has been documented to have activity in vaginitis against *Staphylococcus aureus* and *E. coli* at MICs 0.065 and 0.252% v/v, respectively. In a similar study, anti-*candida* activity was also reported in the range of 0.064-0.2568% v/v.[160] Alpha-terpineol inhibited biofilms

produce by *Candida* spp. after 2 h of exposure at 0.1256% v/v.[157] The activity of α-terpineol in bacterial vaginosis and vulvovaginal candidiasis has been reported against *G. vaginalis* and *C. albicans* at MIC 0.065% and 0.1254% v/v, respectively, by increasing cytokines (interleukins) expressions and NF-κB activation.[124] Alpha-terpineol has shown antifungal activity, along with other constituents.[161] Alpha-terpineol showed prominent inhibitory activity against *Candida albicans* isolates that were resistant to fluconazole with MIC range of 300-3000 µg/mL.[162] A study in 2023, reported to have antibacterial, anti-*candida* and anti-trichomonal activity of tea tree oil due to its main active constituent α-terpineol in a very effective way.[163] Alpha-terpineol is the majorly present in tea tree oil and possesses antibacterial and antifungal activities against ten bacterial isolates and *Candida albicans* isolates. The Rideal-Walker coefficient for α-terpineol was found to be 16, which was the highest among all the constituents of tea tree oil. The anti-inflammatory activity also possessed by α-terpineol in vaginal inflammation occurs in vaginal candidiasis. This study also demonstrated anti-trichomonal activity, concluding that -terpineol effectively combats *Trichomonas vaginalis*.[164,165] Alpha-terpineol is also effective against *Aspergillus niger* and effectively inhibits fungal mycelial growth and spore germination.[165] Alpha-terpineol has antibacterial activity against fifty *Staphylococcus* spp.[166]

Geraniol

Geraniol or 3-7dimethylocta2-6-dien1-ol is a monoterpene found in a mixture of nerol and geraniol (cis and trans forms). It is mostly obtained from *Rosa rubiginosa, Cymbopogon martini, Cymbopogon nardus, Pelargonium graveolens,* and *Monarda fistulosa.* It has a sweet floral or rose-like fragrance, so it is widely used as a fragrance material.[167] It possesses various pharmacological activities, including antitumor, insecticidal, antioxidant, antimicrobial and anti-inflammatory properties. It has efficient antibacterial, antifungal, and antitrichomonal properties for vaginal infections.[168]

Mechanism of action of geraniol

The antimicrobial activity of geraniol has been described against *Staphylococcus aureus* and *E. coli*. Geraniol induces DNA damage and increases osmotic stress in bacterial cells.[169] It disrupts the microbial cell membrane lipid structure, interacts with bacterial cellular components, and makes the bacterial cell more permeable to the outer compounds. It penetrates the cell interior and ultimately limits bacterial cell growth. It also disturbs the membrane efflux pumps in bacterial cells and reduces the level of ergosterol, leading to an alteration in ATP_{ase} activity in the membrane.[170,171] The activity of geraniol has been reported against a broad range of *Candida* and non-*Candida* spp. In fungal cells, it causes impaired iron homeostasis with mitochondrial dysfunction. It also alters the functioning of calcineurin signaling pathway in a fungal cell that is responsible for the antifungal activity of geraniol.[172] In *Candida albicans* spp*.,* membrane disruption is the major mechanism of action of geraniol that affects fungal cells. Geraniol changes the ATP_{ase} activity of the plasma membrane and alters intracellular pH. Geraniol damages DNA repair in *Candida albicans*. Geraniol is a potent inhibitor of hyphae formation in yeast cells. This inhibits biofilm formation.[173] In vaginal infections, geraniol has better anti-trichomonal activity. The treated cells of *Trichomonas vaginalis* showed morphological changes, formation of vacuoles, ribosomal disappearance, and dilation in the rough endoplasmic reticulum. After treatment with geraniol, the protozoan nucleus membrane damages and leads to leakage of cytoplasmic content, accumulation of chromatin in the cytoplasm, disintegrated organelles, and damaged cell membrane, which resulted into cell death.[174,175]

Antimicrobial activity of geraniol

An *in vitro* study reported antifungal activity of geranium oil containing geraniol against *Candida albicans,* and it was found that vaginal washing with geranium oil inhibited fungal growth (IC_{50} 25-26 mg/mL). The study concluded that geraniol is a potent antifungal agent in vaginal candidiasis.[176] Antimicrobial activity of geraniol has been accounted against a wide range of bacteria and fungi. It has remarkable bactericidal activity, with BA_{50} value of 1.5 for *E. coli*. In its gaseous state, it has potent activity against *Staphylococcus aureus*.[177] Researchers have reported the activity

Table 2. Monoterpenes and their antimicrobial activity in vaginal infections (MIC/MBC)

Monoterpene	Source	Family	Activity in vaginal infections	MIC/MBC	Reference
Citral	*Cymbopogon citratus*	*Gramineae*	Anticandida in VVC	0.05%	Onawunmi GO[182]
			S. aureus	0.06%	Naik et al.[183]
			E. coli	0.12%	
Eucalyptol	*Eucalyptus globulus*	*Myrtaceae*	Anticandida in VVC	0.7 mg/mL	Quatrin et al.[184]
			Against Gram-ve bacteria in BV	6.2 mg/mL	Klancnik et al.[185]
			Antitrichomonal activity	12.5 mg/mL	Hashemi et al.[130]
Carvacrol	*Thymus vulgaris*	*Lamiaceae*	Anti-Gardnerella vaginalis activity	0.16 µL/mL	Can Baser[186]
			Anticandida in VVC	10^3 mg/L	Chami et al.[187]
			Antitrichomonal activity	MLC-100 µg/mL	Karami et al.[90]
Thymol	*Thymus vulgaris*	*Lamiaceae*	Anticandida in VVC	0.16 mg/L	Shu et al.[86]
			Staphylococcus aureus	175.5 µg/mL	Sasso et al.[83]
			E. coli	125.23 µg/mL	
			Antitrichomonal activity	2000 µg/mL	Jalili et al.[91]
Eugenol	*Eugenia cariophylata*	*Myrtaceae*	Anticandida in VVC	455.52 mg/L	Chami et al.[187] Fontenelle et al.[188]
			Staphylococcus aureus	128 mg/mL	Marchese et al.[108]
			E. coli	p < 0.001	Filippo et al.[109]
			Antitrichomonal activity	0.156 mg/mL	Jafari et al.[114]
Borneol	*Rosmarinus officinalis*	*Lamiaceae*	Anticandida in VVC	1.2 mg/mL	Chen et al.[189]
			Against bacteria in BV	p < 0.005	Bogavac et al.[126]
α-terpineol	*Origanium vulgare*	*Lamiaceae*	Anti-Gardnerella vaginalis activity	0.06%	Li et al.[118]
			Staphylococcus aureus	6.4 mg/mL	Swamy et al.[190]
			E. coli	5.6 cfu/ mL	Yang et al.[191]
			Anticandida in VVC	0.1254%	
			Antitrichomonal activity	0.06%	Andrine et al.[192]
α-pinene	*Rosmarinus officinalis*	*Lamiaceae*	Anticandida in VVC	3125 µg/mL	Silva et al.[193]
			Against bacteria in BV	p < 0.001	
R-Limonene	*Citrus Limon*	*Rutaceae*	Gardnerella activity	1 µL/mL	Schwiertz et al.[194]
			Staphylococcus aureus	20 mL/L	Han et al.[195]
			Anticandida in VVC	2 µL/mL	
			Antitrichomonal activity	150 µg/mL	Jamshidi et al.[196]
Geraniol	*Cymbopogon nardus*	*Poaecea*	*Staphylococcus aureus*	11200 µg/mL	Bhattamisra et al.[197]
			E. coli	5600 µg/mL	Sharma et al.[198]
			Anticandida in VVC	30-130 µg/mL	de Brum et al.[199]
			Antitrichomonal activity	342.96 µg/mL	
Camphene	*Chrysanth-emum morifolium*	*Asteraceae*	Anticandida in VVC	128 µg/mL 2-	Thakre et al.[200]
			Against bacteria in BV	31.2 µg/mL	de Freitas et al.[201]
Linalool	*Lavendula angustifolia*	*Lamiaceae*	Anti-Gardnerella vaginalis activity	1.25 µL/mL	
			Staphylococcus aureus	12.8 mg/mL	Sousa et al.[202]
			Anticandida in VVC	16-32 µg/mL	Hsu et al.[203]
			Antitrichomonal activity	25.34 µg/mL	Maria et al.[204]
Fenchone	*Foeniculum vulgare*	*Umbelliferae*	Anticandida in VVC	62.51 µg/mL	Bozovic et al.[205]
			Staphylococcus aureus	6.256 µg/mL	Ghasemian et al.[206]
			Antitrichomonal activity	100 µg/mL	Maria et al.[204]

Table 2. Cont...

Monoterpene	Source	Family	Activity in	MIC/MBC vaginal infections	Reference
Trans-anethole	*Foeniculum vulgare*	*Umbelliferae*	Anticandida in VVC	123.50 mg/mL	Dabrowska et al.[207]
			Staphylococcus aureus	6-10%	Kwiatkowski et al.[208]
Menthol	*Mentha piperita*	Lamiaceae	*Staphylococcus aureus*	1.11 mg/mL	Kifer et al.[209]
			Anticandida in VVC	500 µg/mL	Samber et al.[210]
Menthone	*Mentha piperita*	*Lamiaceae*	*Staphylococcus aureus*	3540 µg/mL	Zhao et al.[211]
			Anticandida in VVC	$p < 0.005$	Iraji et al.[212]
β-Myrcene	*Cannabis sativa*	*Cannabiaceae*	Anticandida in VVC	39-78 µg/mL	Cecchini et al.[213]
Terpinen-4-ol	*Origanium vulgare*	*Lamiaceae*	Anti-Gardnerella vaginalis activity	0.13%	
			Anticandida in VVC	0.06%	Mondello et al.[214]
			Antitrichomonal activity	0.003%	Menezes et al.[105]
p-cymene	*Artemesia vulgaris*	*Asteraceae*	Anticandida in VVC	4 mg/mL	Randelovic et al.[215]
			Staphylococcus aureus, E. coli	>8%	Balahbib et al.[216]
			Antitrichomonal activity	>0.01%	Maria et al.[204]
Carvone	*Carum carvi*	*Apiaceae*	Anticandida in VVC	0.312 mg/mL	Mun et al.[217]
			Staphylococcus aureus	500 µg/mL	Moro et al.[218]
			Antitrichomonal activity	72.11 µg/mL	Dominguez et al.[219]
Carvacrol	*Origanium vulgare*	*Lamiaceae*	Anticandida in VVC	256 µg/mL	Lima et al.[220]
			Staphylococcus aureus	400 µg/mL	Rua et al.[221]
α-phellanderene	*Eucalyptus phellandra*	Myrtaceae	Anticandida in VVC	1.7 mL/L	Thangaleela et al.[222]
Geranyl acetate	*Callitris* spp	*Cuprassaceae*	Anticandida in VVC	0.3 mg/mL	Humeriah et al.[223]
			Staphylococcus aureus	2.5 mg/mL	
Nerol	*Cymbopogon flexuosus*	*Poaceae*	Anticandida in VVC	0.77 µL/mL	Wang et al.[224]
			Staphylococcus aureus	40 µg/mL	Togashi et al.[225]
Sabinene	*Quercus ilex*	*Fagaceae*	*Staphylococcus aureus*	6.25 µg/mL	Mahizaan et al.[226]

of geraniol with carvacrol and fluconazole against seven different strains of *Candida albicans*. Geraniol showed a MIC value range 0.5-2 mg/mL for those tested strains. The synergism of geraniol has been reported with fluconazole against resistant fungal species.[177] A study involved antifungal activity of geraniol at the MIC_{90} value was 16 µg/mL and it was recommended that geraniol as a potent anti-*Candida* agent.[178] Another study reported anti-trichomonal activity of geraniol against two isolates of *Trichomonas vaginalis.* Geraniol showed MLC values for TV1 and TV2 isolates as 45 mg/mL and 89.95 mg/mL, respectively. The IC_{50} values were found out to be 22.5 mg/mL and 45 mg/mL, respectively.[174] The antimicrobial activity of rose oil containing geraniol as a major constituent against *Staphylococcus aureus* (methicillin-resistant) and mycelial growth of *Candida* reduced 99 percent of *Staphylococcus aureus* and inhibited the mycelial growth of *Candida albicans* within 1 h of geraniol treatment with an IC_{50} value of 0.00045%.[179] Geraniol's detailed antibacterial and antifungal activity with related mode of action in vaginitis have been well documented.[173] Antifungal activity of nanoemulsion containing geraniol with volatile oil and hydrogel containing essential oil with geraniol has been reported against five strains. The hydrogel containing geraniol showed about 64 times better anti-*Candida* activity.[180] Antibacterial activity of geraniol has been reported for a range of bacteria.[181]

Researchers have reported several monoterpenes, either individually or in combinations, for their intrinsic antimicrobial activities in vaginal infections, as well as their other pharmacological activities. This review reported a collective number of monoterpenes so that they can further be utilized in combinations

to demonstrate their synergistic activities. These antimicrobial activities are against a broad range of micro-organisms, including fungi, bacteria (Gram-positive and Gram-negative bacteria) and other pathogens (Table 2).

CONCLUSION AND FUTURE PROSPECTS

Researchers have extensively studied the vaginal microbiome and its associated infections over the past decade. An imbalance in the vaginal microbiota leads to severe infections. The recurrence of these infections with longer persistence affects a woman in many ways, which can diminish or interfere with her quality of life. The resistance caused by the vaginitis causing microorganisms for conventional drugs led to further research for those antimicrobial agents who can act potently on these microorganisms and their resistant species. Plant secondary metabolites have been mainly focused and examined for their antimicrobial properties as well as other therapeutic effects. Terpenes are the most commonly reported antimicrobial agents in a number of conditions that are associated with microbial infections, including vaginal infections because of their antimicrobial, antioxidant and anti-inflammatory effects. Monoterpenes like linalool, geraniol, eugenol, thymol, limonene, eucalyptol, carvacrol, and terpineol are widely reported to have potent antimicrobial activity in vaginal infections. They have been reported either individually or in many of the combinations present as plant's volatile oils and in combination with conventional drugs. Vaginal infections are caused by some specific strains of microorganisms that attack the vaginal healthy microbiota and cause an imbalance there. For this purpose, certain combinations of plant secondary metabolites are required, which have antimicrobial activities in different dimensions with anti-inflammatory and antioxidant effects. This review summarizes the types of vaginal infections that affect women in today's life and their overlapping persistent effects. Further, this review reported the commonly tested monoterpenes for vaginal infections and their potent effects. We can envision these monoterpenes and their combinations working together in the future due to their strong antimicrobial qualities in vaginal infections, making them the ideal treatment for curing bacterial, fungal, and trichomonal vaginal infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

PS and NA conceptualized the study. SS performed supervision. NA performed visualization. PS and SKY wrote the original draft. HC, SS and KD wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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