Candida Infections

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Candidiasis (oral, vulvovaginal, or systemic bloodstream infections) are important human fungal infections associated with a high global prevalence in otherwise healthy adults but are also opportunistic infections in immunocompromised patients. With the recent discovery of the multidrug resistant—and often difficult to treat—*Candida auris*, as well as the rising costs associated with hospitalisations and the treatment of infections caused by *Candida* species, there is an urgent need to develop effective therapeutics against these pathogenic yeasts. Essential oils have been documented for many years as treatments for different ailments and are widely known and utilised in alternative and complementary therapies, including treating microbial infections.

Candida infections antifungal activity

1. Introduction

Candidiasis is a multifaceted fungal infection caused by commensal fungi *Candida* spp., which reside on the skin, mucosa, and gastrointestinal tract of 30–50% of healthy adults at any given time—with everyone colonised at some point in their lifetime [1]. Under normal host conditions, *Candida* spp. is not usually pathogenic and normally resides in genital and gastrointestinal tracts in healthy humans; however, Candidiasis infection appears when the balance between the fungus, mucosa, and host defence mechanisms is interrupted [2]. *Candida* spp. are known to cause superficial infections (mucosal and cutaneous) and systemic infections. Superficial infections caused by *Candida* spp. vary and present in the form of oral Candidiasis, vaginal Candidiasis, oropharyngeal Candidiasis, onychomycosis, etc., with a relatively high prevalence worldwide (20–25%) [3]. Systemic Candidiasis infections on the other hand are associated with high morbidity and mortality rates as well as increased hospitalisation [4].

Oral Candidiasis is one of the most common human fungal infections, especially in elderly people, human immune deficiency virus (HIV)-positive individuals, or patients receiving radiotherapy treatments [5]. It is reported that the oral carriage rates of *Candida* spp. range from 20–75% in the general population, and up to 95% in HIV patients [6]. Candidiasis that develops in the vagina is commonly called vulvovaginal Candidiasis. In fact, approximately 75% of women suffer from vulvovaginal Candidiasis at least once in their lifetime, and up to 8% of them have recurrent infections [7]. In immunocompromised patients, pathogenic *Candida* spp. can spread through the bloodstream and affect internal organs like the upper gastrointestinal tract, kidney, heart, or brain, leading to systemic Candidiasis with significant morbidity and mortality [6]. According to Fraser et al. [8], systemic Candidiasis carries a mortality rate of 71–79%. Cavayas et al. [9] reported that *Candida* spp. are responsible for the third-highest incidence of isolates from bloodstream infections in neutropenic or immunocompromised hospitalized patients from intensive care units

(ICUs). Moreover, the morbidity and mortality rate associated with Candidiasis is gradually rising because of the increase in the number of high-risk patients and the emergence of new *Candida* spp. and drug resistant strains [10].

Pfaller and Diekema [11] estimated that approximately 62% of all invasive candida infections are caused by *C. albicans*. *C. glabrata* was ranked as the second most common pathogen of candida bloodstream infections in the US with reported prevalence rates of 20–24%. This may be of pertinence as *C. glabrata* is a leading pathogen associated with echinocandin resistance, which is of great concern as echinocandin drugs are the front-line therapeutics for invasive Candidiasis [12]. Some other common *Candida* spp. are *C. tropicalis, C. parapsilosis,* and *C. krusei.* Notably, *C. auris* is a recently emerged *Candida* spp. that was first isolated from a Japanese patient in 2009 and is continuously spreading worldwide [13]. This newly described fungal pathogen can resist several antifungal agents, with some multiple drug resistant (MDR) strains exhibiting resistance to three classes of antifungals (azoles, polyenes, and echinocandins) [14]. *C. auris* can be transmitted person-to-person and it has a high mortality rate (30–60%) in patients who suffered from *C. auris* bloodstream infections [15][16]. Essential oils (EOs) are complex liquid mixtures of volatile and low molecular weight substances that can be extracted from the whole plant or plant parts, such as the leaf, bark, fruits, and flowers of aromatic plants [17][18]. Currently, approximately 3000 EOs have been discovered and about 300 EOs are known to be commercially important [19]. A wide range of effects of EOs from different plant species and botanical families have been reported, including immunomodulatory, psychotropic, acaricide, expectorant, antidiabetic, cancer suppressive, and antibacterial effects [20][21][22]. In addition to these, other effects, such as antifungal properties against various plant and human pathogenic fungi including yeasts, have been reported from numerous EOs [18][23].

Natural compounds found in medicinal plants could be considered as one of the greatest sources for the development of innovative modern medicine [24]. The isolation and characterization of active compounds in EOs is more of a growing science due to the development of new technologies, and detection and characterisation systems such as gas/liquid chromatography and mass spectrometry (GC/LC-MS). Additionally, various available in vitro tests have been utilised to verify the antimicrobial activities of EOs. A significant number of studies have been conducted to evaluate natural compounds of plant origin that are effective but less toxic than those in drugs already in use [25][26].

2. Mode of Action of EOs

Depending on the species, EOs have been shown to comprise a mixture of tens to hundreds of different compounds [27]. Due to this reason, a single EO can possess more than one mechanism of action against a microorganism. Of note, given that every single EO seems to have a wide range of cellular targets (**Table 1**), the probability of the generating new, resistant strains to the EOs as fungicidal agents are low [28].

Table 1. Monoterpenoids and their mode of action upon *Candida* species.

Antifungal Compound	EOs	Mode of Action on *Candida* Species	References
Aldehydes		C=O	
Cinnamaldehyde	Camphor, Cassia, Cinnamon.	ATPase inhibition Induces apoptosis Induction of oxidative stress Reduction of ergosterol biosynthesis	[29][30][31]
Citral	Lemon, Lime, Orange.	Induction of oxidative stress Inhibition of pseudohyphae formation	[32][33]
Cyclic Terpenes		C_6 ring	
α-pinene	Frankincense, Juniper, Pine, Rosemary.	Disruption of cellular membranes Reduced biofilm formation	[34]
β-pinene	Cannabis, Lavender, Mint, Pine.	Disruption of cellular membranes Reduced biofilm formation	[34]
Limonene	Lemon, Lemongrass, Lime, Orange.	Disruption of cellular membranes Induces apoptosis	[35]
p-cymene	Anise, Basil, Camphor, Cumin, Eucalyptus, Oregano, Thyme.	Disruption of cellular membranes Inhibition of germ tube formation	[36]
Phenols		-OH	
Carvacrol	Oregano, Thyme, Wild Bergamot	Binds to sterol components of membranes	[37][38]
Eugenol	Basil, Cinnamon, Clove, Nutmeg.	Altered protein functionality Increases membrane fluidity and permeability Inhibits ergosterol biosynthesis Inhibits proton efflux	[39]
Linalool	Basil, Lavender, Rose, Sage.	Altered protein functionality Increases membrane fluidity and permeability Inhibits proton efflux	[39]
Menthol	Geranium, Mint, Sunflower, Tarragon.	Inhibition of ergosterol biosynthesis	[40]

Antifungal Compound	EOs	Mode of Action on *Candida* Species	References
Thymol	Citrus, Coriander, Oregano, Thyme, Wild Bergamot.	Altered protein functionality Inhibition of ergosterol biosynthesis	[41]

a diverse class of low molecular weight, isoprene-derived molecules that can be further divided into groups depending upon their functional activities. Phenolic terpenes (e.g., carvacrol, eugenol, and thymol) possess an −OH moiety that is able to be transferred to protein structures, thereby altering their integrity and functional capacity [41]. This may be of critical importance when considering the protein content of the cell wall in *Candida* spp., which remains a key factor for adhesion and virulence [42]. However, the most favoured and well-researched target for many phenolic terpenes is the cell membrane structural and regulatory sterol, ergosterol.

Analogous to cholesterol in animal and plant cells, ergosterol has been identified as a primary target for phenolic terpene interactions. As the cardinal sterol component of the plasma membrane of fungal species [18], ergosterol is responsible for the maintenance of cell membrane structure and integrity. Inhibition of ergosterol biosynthesis by numerous monoterpenes, including carvacrol, eugenol, menthol, and thymol, have been demonstrated to affect the fluidity, integrity, and permeability of fungal membranes [29][42]. Here, inhibition of the lanosterol 14-α demethylase enzyme negatively regulates transmethylation processes at position C24 of the sterol sidechain, an effect that has been demonstrated to affect the protein–protein binding functionality of this essential fungal sterol [40].

In addition to the effect phenolic moieties can impose on the structural stability of fungal cells, monoterpenoid alcohols have also been described as having an inhibitory effect on efflux pumps (e.g., carvacrol, thymol) in *Candida* spp. [43][44]. Inhibition of these essential fungal defences could indicate that EO extracts may be viable as an adjunctive therapy, working synergistically alongside current antifungal treatments (e.g., clotrimazole and fluconazole). Similarly, eugenol and linalool have been suggested to inhibit proton efflux channels, which are responsible for regulating cellular pH, and perhaps more importantly, are associated with the electrochemical gradient required for ATP production [39]. Carvacrol, a common component of many EOs, has also been demonstrated to induce temporal changes in both cytosolic and vacuolar pH, leading to a dose-dependent increase in cellular Ca+ and subsequent activation of the target of rapamycin (TOR) stress response pathways [36], which leads to apoptosis [38]. With such multifarious actions being attributed to the phenolic class of terpenoids, it is seemingly the combination of molecules within various EOs that provide continued fungicidal activity.

2.2. Cyclic Terpenes

Cyclic terpenes contain a hydrophobic six-carbon ring that is known to penetrate, disrupt, and increase the fluidity of the cytoplasmic membrane of *Candida* and other fungal species [45]. This particular class of terpenes include α-pinene, β-pinene, limonene, and p-cymene, among others, which are prominent in many EOs. An in silico study of *C. albicans*, conducted by Pinto et al. [36], suggests p-cymene may act primarily as an antagonist to fungal membranes, promoting cellular permeability, the leakage of cytosolic content, and cessation of activity. An alternative mode of action, however, is displayed by limonene, a major constituent of citrus derived EOs, which are noted to induce the apoptotic pathway in *C. albicans* [35].

In addition to the membrane disruption modality displayed by the majority of cyclic terpenes, α-pinene and β-pinene isomers, when examined by Rivas et al. [34], showed a notable reduction in *C. albicans* biofilm formation. However, whether this effect is due to quorum sensing interference [46], interruption of fundamental cellular processes [47], or by other means is still unclear. The diverse mechanisms of fungicidal activity exhibited by this class of phytochemicals could explain the continued susceptibility of *Candida* spp. to EOs and their active components, although much research will be required if the intricacies and efficacies of such biochemical processes are to be fully understood.

2.3. Aldehyde Terpenes

Aldehyde compounds such as cinnamaldehyde and citral are another class of antifungal phytochemicals that are found extensively in the EOs of cinnamon bark and citrus rinds, respectively. Although these molecules have analogous functional moieties, their modes of fungicidal action appear to be diacritic. To illustrate, cinnamaldehyde contains an aromatic ring structure, and in common with other terpenes, exhibits antifungal properties that include dissolution into the hydrophobic domain of cellular membranes, reduction in ergosterol biosynthesis, and inhibition of ATPase and proteinaceous activities [30][31]. Furthermore, the addition of cinnamon-derived EOs to in vitro colonies of *C. albicans* and *C. auris* reveals the inhibition of haemolysin production and reduced hyphae formation [29]. Interestingly, cell wall perturbations and interference with ergosterol processes and production were ruled out as targets for citral. Instead, it has been posited that the inhibition of pseudohyphae and chlamydoconidium may be responsible for the fungicidal effects seen in *C. albicans* [32][33].

As each individual EO can contain varying amounts of active compounds, and each compound may have a similar or unique mode of action against *Candida* cells, future research may involve comparative analysis that assesses the financial and sustainable viability of EOs as modern therapeutics.

3. Activity of EOs against Drug-Resistant *Candida* spp.

Invasive Candida infections pose major health concerns, especially in hospitalised, immunocompromised, or critically ill patients [48][49]. However, there are only four major classes of antifungals in clinical use, which include azoles, polyenes, echinocandins, and pyrimidine analogs [50][51]. For this reason, an intense search for new alternative antifungal compounds is very urgent and necessary.

Previous research has explored the effect of 21 plant essential oils against multidrug resistant *Candida* spp., where it was discovered that *Cymbopogon martini* (lemongrass, LEO), citral, and cinnamaldehyde exhibited great inhibitory activities with MIC ranging from 90–100 µg/mL [52]. Furthermore, these EOs were more effective than fluconazole and amphotericin B. Therefore, the enhanced tolerance to antifungal drugs among *Candida* spp. and the role of biofilm in disease development has necessitated research for new antifungal treatment strategies [52].

A recent study by Jafri and Ahmad [53], which examined the effect of the *Thymus vulgaris* EO (Thyme, TEO) and thymol—its major active compound—on *C. tropicalis* resulted in the discovery that thymol at 0.78–25 µg/mL and

TEO used at the same concentration contributed to the significant reduction of biofilm formation by *C. tropicalis*. Furthermore, the same research showed that, when treated with thymol, the biofilm cells of *C. albicans* showed disaggregation and had deformed shapes. Additionally, there was reduced hyphae formation in *C. tropicalis* biofilms.

As a result of the globally emerging threats of multidrug resistant *C. auris* [54], further research by Hamdy et al. [55] led to the development of novel antifungal drugs that are effective against not only *C. albicans,* but also *C. auris* through the use of cuminaldehyde isolated from the *Calligonum comosum* plant, which has demonstrated broad-spectrum antifungal activities. In this research, new compounds were designed and developed with the incorporation of azoles, whereby the new compounds developed showed significant anti-Candida activities against both *C. auris* and *C. albicans*. This resulted in the formulation of polymeric nanoparticles that possess significantly enhanced activities against *C. albicans* and *C. auris* while maintaining prolonged action and no toxicity at lower concentrations [55].

The enhanced ability of some *Candida* species to form biofilms that promote yeast survival upon exposure to drugs contributes to the acquisition of resistance [56]. According to research by Khan and Ahmad [57], pre-formed biofilms of *C. albicans* showed ≥1024 times increased resistance to antifungal drugs. However, at a concentration of 50–180 µg/mL, oils of *Cymbopogon citratus*, commonly known as west Indian lemongrass, and *Syzygium aromaticum* (clove) inhibited biofilm formation. Here, in the presence of a *C. citratus* EO, the three-dimensional structures of the biofilms produced by both *Candida* species showed deformation. In addition to the drug resistance described earlier, the phase in which the EOs are administered can also determine the type of effect they have on *Candida* species. An example of this was reported by Santomauro et al. [58], whereby the vapour and liquid phases of the *Artemisia annua* EO were analysed against several strains of *Candida* spp. The authors describe that the antifungal activity of *A. annua* is influenced by the type of method adopted, as the inhibitory action of this EO was, in fact, greater in the vapour phase than liquid. This research determined that the average MIC in the liquid phase was 11.88 µL/mL, while the vapour phase was 2.13 µL/mL. However, it was discovered that a strain of *C. glabrata* was more susceptible to the liquid phase than vapour phase. It is also interesting to note that *C. albicans* and *C. dubliniensis* were the most susceptible to vapourised *A. annua,* while *C. parapsilosis* was the least susceptible strain.

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