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Candida Infections and Antifungal Activity of Natural Products on Its Virulence Factors: A Review

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Abstract

Candida infections, particularly candidemia, represent a significant and escalating global health challenge, marked by high incidence and mortality rates, especially among vulnerable populations. The clinical landscape is further complicated by a notable shift towards non-*albicans Candida* (NAC) species, many of which exhibit intrinsic or rapidly acquired resistance to conventional antifungal agents. These resistance mechanisms are diverse, ranging from efflux pump overexpression and target enzyme alterations to the formidable protective capabilities of biofilm formation. Concurrently, *Candida* species employ a sophisticated array of virulence factors, including morphological plasticity, adhesion, secreted hydrolytic enzymes, and intricate immune evasion strategies, all of which contribute to their pathogenicity and ability to persist in the host.

This report comprehensively reviews the potential of natural products as a promising avenue for developing novel antifungal therapies. Diverse classes of natural compounds, including essential oils, terpenoids, polyphenols, flavonoids, alkaloids, coumarins, saponins, and antifungal peptides, have demonstrated potent anti-*Candida* activity. Their mechanisms of action are often multi-targeted, disrupting fungal membranes, inhibiting cell wall synthesis, inducing apoptosis, and crucially, modulating specific virulence factors such as biofilm formation, morphological transitions, adhesion, and enzyme secretion. This multi-pronged attack offers a strategic advantage over traditional single-target drugs, potentially mitigating the development of resistance.

Despite compelling *in vitro* and preclinical *in vivo* evidence, the clinical translation of natural products faces significant hurdles. Challenges include poor bioavailability, lack of standardization, potential toxicity concerns, and complex drug-drug interactions. Overcoming these limitations necessitates a concerted effort involving advanced analytical techniques, innovative drug delivery systems, structural modification, and rigorous clinical trial design. The future of antifungal therapy may lie in harnessing these natural compounds, not only as direct fungicidal agents but also as anti-virulence modulators or synergistic partners with existing drugs, thereby offering a more resilient and effective approach to combat the growing burden of *Candida* infections.

1. Introduction to *Candida* Infections: A Global Health Challenge

Candida species are opportunistic fungal pathogens that pose a substantial threat to global public health, particularly in healthcare settings. Infections caused by these fungi, ranging from superficial mucocutaneous candidiasis to life-threatening invasive candidemia, are a growing concern due to their increasing incidence, high mortality rates, and the escalating problem of antifungal drug resistance. Understanding the epidemiology, prevalent species, and mechanisms of resistance is paramount to developing effective management and treatment strategies.

1.1. Epidemiology and Burden of Candidiasis

Candidemia, a bloodstream infection caused by *Candida* fungi, stands as one of the most common bloodstream infections within the United States, with an estimated 25,000 cases reported annually. Surveillance data from 2017 to 2021 reveal an overall candidemia incidence of approximately 7.4 cases per 100,000 population, reaching its highest point at 7.9 cases per 100,000 in 2021. The severity of these infections is underscored by the high mortality rates; the all-cause in-hospital mortality rate for candidemia was 32.6% during this period, notably increasing from 26.8% in 2019 to 36.1% in 2021. On a global scale, an estimated 1.565 million individuals contract

Candida bloodstream infections or invasive candidiasis each year, tragically leading to approximately 995,000 deaths, representing a crude mortality rate of 63.6%.

Certain demographic groups bear a disproportionately high burden of candidemia. Patients aged 65 years and older constitute 42.2% of cases, with an incidence rate of 22.7 per 100,000 in 2021, while infants younger than one year old, though representing 1.7% of cases, exhibit a significant incidence of 8.0 per 100,000. Males generally experience a higher incidence (8.7 per 100,000) compared to females (7.0 per 100,000). Racial disparities are particularly pronounced, with Black or African American patients experiencing a candidemia incidence of 12.8 per 100,000 population, nearly twice as high as non-Black patients (5.6 per 100,000).

These demographic patterns, coupled with the increasing prevalence of healthcare-onset infections and comorbidities, illuminate a complex interplay of host susceptibility, vulnerabilities within the healthcare system, and evolving public health challenges. The disproportionate impact observed in Black patients, for instance, extends beyond simple biological predisposition, strongly suggesting the influence of underlying systemic health disparities, unequal access to quality healthcare, and socioeconomic factors that collectively heighten infection risk and worsen outcomes. This highlights a critical need for public health interventions that address the broader social determinants of health.

Common underlying conditions significantly predispose individuals to candidemia. Diabetes is the most frequent comorbidity, present in 36.2% of cases and increasing to 38.0% by 2021.

Other prevalent conditions include chronic kidney disease (25.6%), malignancy (24.3%), and chronic lung disease (22.1%). The increasing percentage of cases classified as healthcare-onset, rising from 52.2% in 2017 to 58.0% in 2021, and the higher proportion of cases involving an ICU stay (44.9% in 2021 versus 38.3% in 2017), underscore the nosocomial nature of severe candidemia. Critically ill patients in intensive care units, frequently equipped with invasive medical devices such as catheters, central venous lines, and ventilators, face a heightened risk as these devices create direct entry points for

Candida into the bloodstream. This is not merely a correlation but a direct causal pathway for invasive infections in an already vulnerable patient population. Furthermore, the association with COVID-19 is notable, with cases increasing from 10.4% in 2020 to 17.7% in 2021. This connection reflects the extensive immune dysregulation and prolonged hospitalization often associated with severe COVID-19, which further increases candidemia risk.

Beyond the profound human toll, invasive *Candida* infections impose a substantial economic burden on healthcare systems, requiring an estimated 3 to 13 additional days of hospitalization and incurring healthcare costs ranging from $6,000 to $29,000 per case. The collective data paints a picture of candidemia as a disease rooted in vulnerability, where compromised host defenses, intensive medical interventions, and systemic health disparities converge to create a significant public health burden with far-reaching social implications.

Table 1: Global Burden and Key Characteristics of Candidemia

Characteristic	Data (2017-2021, unless specified)
Annual Incidence (US)	~25,000 cases annually
Overall Candidemia Incidence (US)	7.4 cases per 100,000 population (peak 7.9 in 2021)
Global Incidence (Invasive Candidiasis)	~1.565 million people annually
All-Cause In-Hospital Mortality Rate	32.6% (increased from 26.8% in 2019 to 36.1% in 2021)
Global Deaths (Invasive Candidiasis)	~995,000 deaths annually (63.6% mortality)
Most Prevalent *Candida* Species	*C. albicans* (37.1%), *C. glabrata* (30.4%), *C. parapsilosis* (13.5%)
Overall Species Distribution	*C. albicans* (leading cause), Non-*albicans* species (approx. two-thirds of cases)
High-Risk Age Groups	≥65 years (42.2% of cases, incidence 22.7); <1 year (1.7% of cases, incidence 8.0)
Sex Disparity	Males (incidence 8.7) > Females (incidence 7.0)

Characteristic	Data (2017-2021, unless specified)
Racial/Ethnic Disparity	Black patients (incidence 12.8) nearly twice as high as non-Black (5.6)
Common Underlying Conditions	Diabetes (36.2%), Chronic Kidney Disease (25.6%), Malignancy (24.3%), Chronic Lung Disease (22.1%)
Healthcare-Onset Cases	Increased from 52.2% (2017) to 58.0% (2021)
ICU Stay Association	Increased from 38.3% (2017) to 44.9% (2021)
Medical Devices as Entry Points	Surgical wounds, catheters, central venous lines, ventilators
COVID-19 Association	Increased from 10.4% (2020) to 17.7% (2021)
Estimated Healthcare Costs (per case)	3-13 additional days of hospitalization; $6,000-$29,000

1.2. Prevalent *Candida* Species and Emerging Trends

While *Candida albicans* has historically been, and largely remains, the predominant cause of candidemia, accounting for 37.1% of cases between 2017 and 2021, a significant epidemiological shift has been observed. Non-

Candida albicans Candida (NAC) species are increasingly prevalent, now responsible for approximately two-thirds of all *Candida* infections. This changing landscape fundamentally alters the clinical approach to candidiasis.

The five most common *Candida* species responsible for up to 95% of bloodstream infections in the United States include *C. albicans*, *C. glabrata* (now officially *Nakaseomyces glabratus*), *C. parapsilosis*, *C. tropicalis*, and *C. krusei* (now *Pichia kudriavzevii*). Among these,

C. glabrata is the second most frequently isolated species, accounting for 30.4% of cases, and in some geographic regions, it has even surpassed *C. albicans* as the leading cause of candidemia.

This increasing prevalence of NAC species is not merely a statistical observation but a critical challenge to current antifungal treatment paradigms. Many NAC species exhibit higher intrinsic resistance to common antifungals. For example, *C. glabrata* is known for its high levels of fluconazole resistance, and *C. krusei* is intrinsically resistant to fluconazole. The emergence of multidrug-resistant

Candida auris (*Candidozyma auris*) has become a particularly urgent global concern due to its high transmissibility and resistance to multiple antifungal classes. This shift means that empirical antifungal therapy, often primarily targeting

C. albicans, may prove ineffective, leading to treatment failures, prolonged hospitalization, and increased mortality. Consequently, accurate and timely species identification has become even more critical for guiding appropriate and effective therapy.

The rise of NAC species is linked to several factors, including the widespread use of antibiotics and antifungals, increased reliance on intensive care, exposure to foreign bodies such as post-surgical catheters, and a growing population of immunosuppressed and chronically ill patients. This suggests that human medical practices, particularly the overuse of antifungals, are driving this epidemiological change by selecting for more resistant strains. Furthermore, "rare" or atypical

Candida species, such as *Candida guilliermondii* (*Meyerozyma guilliermondii*), *C. lusitaniae* (*Clavispora lusitaniae*), *C. norvegensis* (*Pichia norvegensis*), *C. inconspicua* (*Pichia inconspicua*), and *C. kefyr* (*Kluyveromyces marxianus*), are also increasingly recognized as significant contributors to fungal infections, especially in immunocompromised individuals. The accurate identification of these atypical species is crucial for appropriate treatment, yet it can be challenging with standard laboratory methods, further complicating timely and effective management. This evolving epidemiological landscape underscores the dynamic nature of

Candida infections and the urgent need for a broader diagnostic approach and diversified antifungal strategies.

1.3. Antifungal Resistance: Mechanisms and Clinical Implications

Antifungal resistance represents a formidable and growing challenge in the management of *Candida* infections, exacerbated by the limited number of antifungal classes currently available. While approximately 6% of all

Candida bloodstream isolates tested by the CDC exhibit resistance to fluconazole, a proportion that has remained relatively stable over the past two decades, the overall rates of resistance to azole drugs are increasing annually. This trend is particularly pronounced for

C. tropicalis, which shows the highest resistance rate to azoles, and for *C. glabrata*, known for its high levels of fluconazole resistance.

Antifungal resistance can be broadly categorized as either intrinsic, meaning it is a natural characteristic of a particular fungal species (e.g., *C. krusei*'s natural resistance to fluconazole or *C. glabrata*'s inherent fluconazole resistance), or acquired, developing in response to prior

exposure to an antifungal agent. The detailed molecular mechanisms underlying this resistance are complex and multifactorial, reflecting the remarkable adaptability of

Candida species under drug pressure.

Mechanisms of Azole Resistance: Azoles are fungistatic drugs that inhibit the fungal cytochrome P450 14α-lanosterol demethylase (Erg11), an enzyme crucial for ergosterol biosynthesis. Resistance primarily arises through:

- **Overexpression of Efflux Pumps:** This is a major and highly common mechanism, involving two main classes of transporters: ATP-binding cassette (ABC) transporters (encoded by *CDR1* and *CDR2* genes) and major facilitator superfamily (MFS) transporters (encoded by the *MDR1* gene). These pumps actively extrude azole drugs from the fungal cell, thereby reducing intracellular drug concentrations to sub-inhibitory levels. Upregulation of

  CDR1 and *CDR2* is the most frequent mechanism observed in clinical isolates of *C. albicans*, with their expression often regulated by gain-of-function mutations in the transcription factor Tac1. While

  MDR1 overexpression is less common in *C. albicans*, it is specifically implicated in fluconazole resistance and is controlled by the transcription factor Mrr1. In

  C. glabrata, overexpression of ABC transporters (*CgCDR1*, *CgCDR2*, and *CgSNQ2*) and MFS efflux pumps constitutes a primary mechanism of azole resistance. Similarly,

  C. tropicalis has been reported to upregulate *CtMDR1* and *CtCDR1* as a resistance strategy.

- **Alterations in Target Enzyme (*Erg11*):** Mutations within the *ERG11* gene, which encodes the azole target enzyme 14α-lanosterol demethylase, can lead to a reduced affinity of the enzyme for azoles, preventing effective drug binding. Alternatively, overexpression of the

  ERG11 gene can result in an increased amount of the target enzyme, overwhelming the inhibitory effect of the drug. Specific point mutations, such as D116E and E266D in

  C. albicans, or Y132F, S154F, and D275V in *C. tropicalis*, are well-documented associations with azole resistance.

- **Alterations in Ergosterol Biosynthesis Pathway:** Mutations in other genes involved in ergosterol biosynthesis, such as *ERG3* (encoding sterol Δ5,6-desaturase), can lead to the accumulation of non-toxic sterol intermediates. This bypass mechanism reduces the fungus's reliance on ergosterol, thereby diminishing the effectiveness of azoles that target this pathway.

Mechanisms of Echinocandin Resistance: Echinocandins are fungicidal drugs that inhibit β-(1,3)-glucan synthase, an enzyme critical for the synthesis of β-(1,3)-glucan, a major component of the fungal cell wall. Resistance to echinocandins is primarily attributed to point mutations in the *FKS1* gene, and to a lesser extent *FKS2*, which encode the catalytic subunits of β-(1,3)-glucan synthase. These mutations result in amino acid substitutions in conserved "hot spot" regions of the enzyme, rendering it significantly less sensitive to the drug.

Mechanisms of Polyene Resistance (e.g., Amphotericin B): Polyenes bind to ergosterol in the fungal membrane, disrupting its integrity. Resistance to polyenes is less common but can arise from mutations in the *ERG3* gene, leading to a reduced concentration of ergosterol in the fungal membrane. Fungi may also increase catalase activity to decrease their susceptibility to oxidative damage induced by polyenes. Additionally, an increased cell wall thickness, potentially due to high β-1,3-glucan levels, may act as a protective response against amphotericin B.

Mechanisms of Flucytosine Resistance: Flucytosine is a pyrimidine analogue that inhibits fungal DNA and RNA synthesis. Resistance typically correlates with mutations in the enzyme uracil phosphoribosyltransferase (Fur1p) or cytosine permease (Fcy2), which prevent the conversion of the drug into its active toxic form within the fungal cell.

Biofilm Formation: Beyond specific molecular alterations, *Candida* biofilms represent a major mechanism of drug tolerance. Biofilms are communities of adherent cells encased in a self-produced extracellular matrix (ECM). These structures exhibit innate resistance to multiple drug classes, capable of withstanding antifungal concentrations up to 1000-fold higher than their planktonic (free-floating) counterparts. This extreme resistance is multifactorial, involving increased efflux pump activity, the physical barrier provided by the ECM (composed of β-glucan and extracellular DNA), and the engagement of stress response pathways. The ECM shields enclosed cells from both antimicrobial agents and the host's immune system, significantly increasing cell survival.

Other Resistance Mechanisms: *Candida* can also develop resistance through altered chromosome abnormalities, such as loss of heterozygosity, increased chromosome copy number, or aneuploidies. Furthermore, the fungus can exploit various cellular tolerance pathways, including the cAMP-PKA, Ca2+-calmodulin-calcineurin, and Heat Shock Protein 90 (Hsp90) pathways, to survive and adapt under antifungal exposure.

The detailed molecular mechanisms of antifungal resistance reveal a common evolutionary strategy across *Candida* species: altering drug targets, enhancing drug efflux, and adapting cellular processes to survive drug pressure. This demonstrates that resistance is not a random phenomenon but a direct consequence of the selective pressure exerted by widespread antifungal use, leading to a pressing need for novel drug targets and combination therapies that circumvent these established resistance pathways. The consistent emergence of efflux pump overexpression and target modifications across species (e.g., *CDR1/CDR2* in *C. albicans*, *C. glabrata*, *C. tropicalis*) clearly indicates this strong selective pressure. This often leads to cross-resistance, where resistance to one drug compromises an entire class of antifungals, significantly narrowing treatment options. The role of biofilms as a major contributor to resistance underscores the inadequacy of current drug concentrations for device-related infections, often necessitating surgical intervention, as pharmaceutical options are frequently insufficient to eradicate these infections. This highlights a critical gap in current antifungal strategies. The fact that current antifungals primarily target ergosterol or cell wall glucan means that resistance mechanisms often converge on these pathways, necessitating the discovery of drugs with novel mechanisms of action that target different fungal vulnerabilities.

Table 2: Mechanisms of Antifungal Resistance in Major *Candida* Species

Antifungal Class	*Candida* Species	Primary Resistance Mechanisms	Key Genes Involved
Azoles	*C. albicans*	Overexpression of efflux pumps (ABC & MFS); Alterations (mutation/overexpression) in target enzyme (Erg11)	*CDR1*, *CDR2*, *MDR1*, *ERG11*, Tac1, Mrr1
	C. glabrata	Overexpression of efflux pumps (ABC & MFS); Alterations in target enzyme (Erg11)	*CgCDR1*, *CgCDR2*, *CgSNQ2*, *CgERG11*
	C. tropicalis	Alterations (mutation/overexpression) in target enzyme (Erg11); Overexpression of efflux pumps (ABC & MFS); Alterations in ergosterol biosynthesis (Erg3)	*CtERG11*, *CtMDR1*, *CtCDR1*, *ERG3*, Upc2, Mrr1, Tac1
	C. krusei	Intrinsic resistance (due to altered Erg11)	*ERG11*
Echinocandins	*C. albicans*, *C. glabrata*, *C.*	Point mutations in β-(1,3)-glucan synthase	*FKS1*, *FKS2*

Antifungal Class	*Candida* Species	Primary Resistance Mechanisms	Key Genes Involved
	parapsilosis, *C. tropicalis*		
Polyenes	*C. albicans*, *C. tropicalis*	Alterations in ergosterol biosynthesis (reduced ergosterol); Increased catalase activity; Increased cell wall thickness	*ERG3*
Flucytosine	*C. albicans*, *C. tropicalis*	Mutations in enzymes involved in drug activation/uptake	*Fur1p*, *FCY2*
Biofilm Formation	All major *Candida* species (esp. *C. albicans*)	Extracellular matrix (physical barrier); Increased efflux pump activity; Altered metabolic activity; Stress response pathways	*CDR1*, *CDR2*, *MDR1*, β-glucan, extracellular DNA
Chromosomal Abnormalities	*C. albicans*	Loss of heterozygosity; Increased chromosome copy number; Aneuploidies	Chr5 rearrangements
Tolerance Pathways	*C. albicans*	Activation of stress response pathways	cAMP-PKA, Ca2+-calmodulin-calcineurin, Hsp90

2. *Candida* Virulence Factors: Orchestrating Pathogenicity

The ability of *Candida* species to cause disease is not solely dependent on their capacity to grow within a host but is intricately linked to a diverse array of virulence factors. These attributes enable the fungus to colonize various host niches, evade immune surveillance, invade tissues, and establish persistent infections, often in the face of antimicrobial therapy. Understanding these virulence mechanisms is crucial for identifying novel therapeutic targets that aim to disarm the pathogen rather than solely focusing on its eradication.

2.1. Morphological Plasticity and Tissue Invasion

Candida albicans exhibits a remarkable characteristic known as morphological plasticity, which is central to its pathogenic life cycle. This involves its ability to reversibly switch between distinct cellular forms: yeast, pseudohyphae, and true hyphae. This morphogenic transition is critical for its ability to breach host mucosal barriers and establish invasive disease.

Typically, the yeast form is associated with colonization of superficial commensal niches, such as the gastrointestinal tract or mucosal surfaces. In contrast, the elongated, filamentous hyphal form is generally regarded as the invasive form, enabling

C. albicans to penetrate host barriers and gain access to deep-seated tissues. Environmental cues within the host play a pivotal role in triggering this switch. Factors such as host temperature (above 37°C), alkaline pH, the presence of serum, high carbon dioxide concentrations, and specific nutrient availability (e.g., a lack of nitrogen or carbon in the presence of N-acetylglucosamine) are all known to enhance filamentation.

The significance of this yeast-to-hyphae transition is underscored by the observation that *C. albicans* strains engineered to be non-filamentous are often avirulent. However, a critical nuance arises when considering non-

albicans Candida (NAC) species. The successful pathogenicity of species like *C. glabrata* and *C. auris*, which are largely unable to form true hyphae, presents an apparent contradiction to the idea that filamentation is a universal prerequisite for *Candida* pathogenesis. This observation implies that while morphological plasticity is a major virulence factor for

C. albicans, it is not the sole or universal determinant of pathogenicity across the entire *Candida* genus. NAC species must therefore employ other virulence factors more effectively to compensate for their lack of filamentation, such as strong adhesion capabilities, efficient immune evasion strategies, or the secretion of potent enzymes. This understanding is crucial because it suggests that a "one-size-fits-all" therapeutic approach primarily targeting filamentation might be effective against

C. albicans but would prove ineffective against many increasingly prevalent NAC species. Therefore, research and drug development must consider the diverse and species-specific virulence arsenals employed by *Candida* pathogens. Indeed, studies have shown that for *C. albicans*, the ability to switch *between* the yeast and filamentous forms is most critical for effective virulence, rather than a single morphogenic state itself. This dynamic interconversion allows the fungus to adapt to different host environments and optimize its invasive potential.

2.2. Biofilm Formation and Drug Tolerance

Candida species, particularly *C. albicans*, possess a remarkable ability to form robust biofilms on both medical devices, such as catheters and central venous lines, and various host tissues. These structured communities of adherent cells represent a major cause of persistent and difficult-to-treat infections.

A defining characteristic of *Candida* biofilms is their encapsulation within a self-produced extracellular matrix (ECM). This polymeric material, primarily composed of β-glucan and

extracellular DNA, acts as a physical barrier, effectively shielding the embedded fungal cells from both antifungal drugs and the host's immune defenses. The extreme drug tolerance conferred by

Candida biofilms is profound; biofilm-associated cells can withstand antifungal concentrations up to 1000-fold higher than their planktonic (free-floating) counterparts. This extreme resistance is multifactorial, involving not only the physical barrier of the ECM but also increased activity of drug efflux pumps, altered metabolic states (such as a switch from glycolysis to gluconeogenesis), and the activation of various stress response pathways within the biofilm community.

Mature biofilms are complex, dense networks comprising yeasts, hyphae, and pseudohyphae, interconnected by the polymeric ECM. Within this structure, water channels facilitate the diffusion of nutrients from the environment to the deeper layers of the biomass and allow for efficient waste elimination. Biofilm formation is not merely a passive aggregation but an active defense mechanism against nutritional and environmental stress, involving the coordinated expression of numerous transcriptional factors and specific genes.

The extreme drug tolerance conferred by *Candida* biofilms creates a critical therapeutic challenge that often renders conventional antifungals ineffective. This frequently necessitates invasive procedures, such as the surgical removal of infected medical devices, because systemic concentrations of conventional antifungals, even at maximum tolerated doses, are often insufficient to eradicate biofilm infections. This highlights a significant gap in current antifungal strategies. The unique mechanisms of biofilm resistance, including the protective ECM and altered metabolic states, mean that drugs effective against planktonic cells may not be effective against biofilms. This necessitates a paradigm shift in drug discovery towards agents specifically designed to penetrate and disrupt biofilm structures, or to prevent their formation altogether. Biofilms are therefore not merely a virulence factor but a "super-resistance" mechanism that fundamentally undermines conventional antifungal therapy, emphasizing the urgent need for innovative solutions.

2.3. Adhesion and Host Colonization

The initial step in *Candida* pathogenicity involves successful adhesion to host surfaces. Cell surface-associated adhesin proteins play a crucial role in *C. albicans*'s ability to colonize host tissues, undergo cellular morphogenesis, and develop robust biofilms, ultimately leading to infection. These adhesins, such as Als1-7, Als9, and Hwp1, are responsible for the fungus's attachment to host cells.

Adhesion is the foundational step for *Candida* pathogenicity, enabling initial colonization and subsequent progression to more complex virulence strategies like biofilm formation and tissue invasion. Without successful adhesion, *Candida* cannot establish a foothold within the host. Once adhered, the fungus can then initiate other critical virulence processes, such as

morphological switching to the invasive hyphal form to penetrate tissues, and the formation of biofilms to establish persistent, drug-tolerant communities. Therefore, successful adhesion is a prerequisite for the subsequent, more severe manifestations of candidiasis. This makes targeting adhesion mechanisms a highly attractive strategy for prophylactic or early-stage therapeutic interventions, aiming to prevent the establishment of infection before it becomes resistant or invasive.

2.4. Secreted Hydrolytic Enzymes

Candida species possess an extensive repertoire of virulence factors, among which extracellular hydrolases are particularly significant. These enzymes contribute substantially to fungal virulence by facilitating host invasion and the spread of infection within the host.

Secreted aspartic proteinases (Saps) are the most extensively studied and discussed hydrolytic enzymes produced by *C. albicans*. These enzymes act as molecular "weapons," contributing to host tissue invasion by directly digesting or destroying host cell membranes and degrading various host surface molecules. Beyond their role in tissue degradation, Saps also play a crucial part in evading host immune defenses by attacking immune cells and molecules, thereby neutralizing components of the host's protective mechanisms.

C. albicans is particularly noted for its high hydrolytic activity and broad substrate specificity, indicating a highly adaptable and aggressive invasive strategy. Other hydrolytic enzymes, such as phospholipases (PL) and lipases (Lip), are also produced by

Candida, although their precise involvement in virulence is less comprehensively understood.

C. albicans, in particular, is known to produce numerous lipases, suggesting a broad-lipolytic activity that may contribute to its persistence and virulence in human tissues.

Secreted hydrolytic enzymes, particularly Saps, are not merely involved in nutrient acquisition; they are active destructive agents. By enzymatically breaking down host barriers (e.g., cell membranes, surface molecules) and neutralizing immune components, they directly facilitate tissue invasion and immune evasion. This direct enzymatic assault on the host enables the fungus to spread and establish infection. Their broad specificity means they can attack a wide range of host targets, making *C. albicans* particularly adept at causing invasive disease. Targeting these enzymes with specific inhibitors could effectively disarm the fungus without necessarily killing it, potentially reducing the selective pressure for resistance development.

2.5. Immune Evasion Strategies

Candida species have co-evolved with their human hosts, developing sophisticated and multi-layered mechanisms to evade the host's immune system. These strategies are crucial for their survival, persistence, and ability to cause invasive infections.

One prominent mechanism involves evading complement-mediated host defense, a critical component of innate immunity. *Candida* achieves this through several tactics, including the masking of immune-stimulatory cell wall components (e.g., β-1,3-glucan masking with mannoproteins), proteolytic cleavage and inhibition of complement proteins, recruitment of host complement regulators, and acquisition of host proteins that interfere with complement activation. This masking of pathogen-associated molecular patterns (PAMPs) hinders recognition by host pattern recognition receptors (PRRs) on immune cells, leading to a poor cytokine response.

Secreted aspartic proteinases (Saps), discussed previously for their role in tissue invasion, also contribute significantly to immune evasion. These proteases degrade various components of the innate immune system, including salivary lactoferrin, lactoperoxidase, cathepsin D, complement proteins, interleukin-1β, and α2-macroglobulin. This enzymatic degradation directly compromises the host's defense mechanisms.

Candida species also employ diverse phagocyte escape strategies. In *C. albicans*, filamentation of phagocytized cells can lead to mechanical perforation of immune cells, allowing the pathogen to escape. This process can also induce pyroptosis, a programmed cell death pathway in macrophages, partially dependent on hyphal morphogenesis. Other species, such as

Nakaseomyces glabrata, utilize different tactics, including intracellular survival and replication within macrophages. *N. glabrata* is also known to manipulate phagolysosome maturation, a strategy shared with *C. parapsilosis* and *Pichia kudriavzevii*, allowing them to survive within these immune cells.

The multidrug-resistant *Candida auris* exhibits particularly concerning immune evasion capabilities. It can evade the immune response from neutrophils, which are vital in controlling fungal infections. Studies show reduced uptake of *C. auris* by human monocyte-derived macrophages, and it stimulates minimal production of pro-inflammatory cytokines such as TNFα, IL-6, IL-1β, and IL-10 compared to other *Candida* species. This reduced inflammatory response may allow the infection to progress with fewer overt signs, delaying diagnosis and treatment. This "stealth" pathogenicity is a significant clinical challenge.

Furthermore, *Candida* species activate robust antioxidant responses (e.g., via the Yap1 protein and Skn7 regulator) to counteract the reactive oxygen species (ROS) produced by phagocytic cells as part of the host's oxidative burst defense. Metabolic reprogramming, such as adapting

to nutrient-limited environments within phagocytes and utilizing biotin restriction, also contributes to their persistence and evasion.

Candida's immune evasion mechanisms are highly sophisticated and multi-layered, often intertwined with other virulence factors. For instance, Saps contribute to both tissue invasion and the degradation of immune components, while filamentation aids in both tissue invasion and phagocyte escape. This intricate network of evasion strategies is a primary reason for persistent and invasive infections, highlighting that effective antifungal therapies must not only kill the fungus but also disrupt its ability to hide from and manipulate the host immune system. The sheer variety of evasion mechanisms indicates a highly evolved pathogen that attacks the immune system from multiple angles (recognition, killing, inflammatory response). This implies that a successful antifungal strategy might need to combine direct fungicidal action with immune-modulating or anti-virulence approaches that restore host recognition and response, moving beyond simply killing the pathogen to re-establishing host control.

3. Natural Products as Antifungal Agents: Classes, Sources, and Mechanisms of Action

The escalating crisis of antifungal resistance and the limited arsenal of conventional drugs have intensified the search for novel therapeutic agents. Natural products, with their vast chemical diversity and evolutionary optimization for biological activity, represent a promising source of new anti-*Candida* compounds. These compounds often exhibit multi-targeted mechanisms of action, offering potential advantages over single-target synthetic drugs.

3.1. Essential Oils and Terpenoids

Essential oils (EOs) are complex mixtures of volatile organic compounds extracted from plants, renowned for their diverse biological activities, including potent antifungal properties. Common sources of EOs with anti-*Candida* activity include lemon balm (*Melissa officinalis*), oregano (*Origanum vulgare*), lavender (*Lavandula stoechas*), mint (*Mentha* × *piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*). Other notable sources include tea tree (

Melaleuca alternifolia), cinnamon (*Cinnamomum zeylanicum*), clove (*Eugenia caryophyllata*), and geranium (*Pelargonium graveolens*).

The antifungal activity of EOs is largely attributed to their primary constituents, the terpenoids. Key terpenoid compounds exhibiting anti-*Candida* effects include linalool, citral, eugenol, citronellal, linalyl acetate, benzyl benzoate, geraniol, geranyl acetate, citronellol, D-limonene, menthol, farnesol, nerolidol, bisabolol, carvacrol, and thymol.

The antifungal mechanisms of these compounds are diverse and often multi-targeted:

- **Membrane Disruption:** Many terpenoids are lipid-soluble, allowing them to readily integrate into fungal cell membranes. They interfere with ergosterol biosynthesis, a crucial component of the fungal membrane (e.g., citral, eugenol, linalool), and directly disrupt membrane integrity, leading to increased permeability, leakage of essential intracellular components (such as ions like H+ and Ca2+), and ultimately, cell death. Eugenol, for instance, makes the

  C. albicans cell membrane permeable in a dose-dependent manner.

- **Cell Cycle Arrest:** Specific terpenoids can arrest *C. albicans* cells at different phases of the cell cycle, ultimately leading to apoptosis. For example, linalool and linalyl acetate cause arrest at the G1 phase, while citral and citronellal halt cells at the S phase, and benzyl benzoate at the G2-M phase.
- **Reactive Oxygen Species (ROS) Induction:** Some terpenoids, such as geraniol, induce mitochondrial depolarization, leading to increased intracellular ROS levels. This oxidative stress causes damage to fungal DNA and plasma membranes, contributing to apoptosis.
- **TOR Pathway Inhibition:** Carvacrol and thymol have been shown to disrupt the TOR (Target of Rapamycin) signaling pathway, which is critical for fungal growth and nutrient sensing.
- **Enzyme Inhibition:** Lemon extracts, containing terpenoids like citral and limonene, have been reported to disrupt cell membrane integrity and suppress fungal enzymes.

The diverse mechanisms of action observed for essential oils and terpenoids, particularly their ability to disrupt fungal membranes, interfere with ergosterol synthesis, induce cell cycle arrest, and generate ROS, highlight a significant advantage over conventional antifungals that often target a single pathway. Conventional antifungals, such as azoles and echinocandins, typically target a single, specific fungal pathway (ergosterol biosynthesis or cell wall glucan synthesis). While initially effective, this single-target specificity creates strong selective pressure for the fungus to develop resistance via mutations or efflux pumps in that specific pathway. Natural products, by often acting on multiple targets or pathways simultaneously, make it significantly harder for the fungus to develop resistance through a single compensatory mechanism. This multi-target approach could lead to more durable and effective antifungal therapies, especially against multidrug-resistant strains.

3.2. Polyphenols and Flavonoids

Polyphenols and flavonoids are broad classes of natural compounds widely distributed in the plant kingdom, recognized for their diverse pharmacological properties, including significant anti-*Candida* activity. Polyphenols are abundant in black tea (catechins and theaflavins) , green tea , and

Curcuma longa (curcuminoids, notably curcumin I, II, and III). Flavonoids, a subgroup of polyphenols, are commonly found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Specific examples include luteolin, quercitrin, isoquercitrin, rutin, quercetin, catechin, and myricetin.

The antifungal mechanisms of polyphenols and flavonoids are multifaceted:

- **Cell Wall and Membrane Disruption:** Flavonoids interfere with the synthesis and assembly of fungal cell wall components, such as 1,3-β-D-glucan, leading to cell wall breakdown and lysis. They also directly damage the fungal cell membrane, increasing permeability and causing leakage of essential intracellular components like DNA and proteins. Myricetin, a flavone derivative, has been shown to impair cell wall integrity and significantly increase membrane permeability in

  C. albicans. Black tea polyphenols cause considerable cell wall damage in

  C. albicans.

- **Ergosterol Synthesis Interference:** Flavonoids can inhibit enzymes involved in ergosterol biosynthesis, leading to a depletion of ergosterol in the cell membrane. This depletion causes membrane instability and damage, ultimately resulting in fungal cell death.
- **Reactive Oxygen Species (ROS) Generation:** The chemical structure of many flavonoids enables them to generate ROS within the yeast cell. These ROS cause oxidative damage to critical cellular components, including membrane lipids, proteins, and DNA, contributing to cell death. Curcumin I, for instance, is known to generate ROS, which triggers apoptosis in

  C. albicans.

- **Mitochondrial Dysfunction:** Flavonoids can disrupt mitochondrial function in fungal cells, leading to decreased energy production and activation of apoptotic pathways. This involves inhibiting electron transport chain complexes and inducing mitochondrial membrane depolarization.
- **Cell Division Inhibition:** Some flavonoids interfere with fungal mitosis, causing cell cycle arrest.
- **Proteasome Inactivation:** Tea polyphenols have been observed to inhibit proteasome activity in *C. albicans*, contributing to cellular metabolic and structural disruptions that impede biofilm formation and maintenance.

The demonstrated synergistic activity of polyphenols (e.g., curcumin) and flavonoids with conventional antifungals (azoles, polyenes) is a highly significant finding. Antifungal resistance is a major challenge, and the development of new drugs is slow. If natural products

can potentiate existing drugs, it provides a crucial strategy to overcome resistance by reducing the minimum inhibitory concentrations (MICs) of resistant strains, potentially making previously ineffective drugs work again. This synergy can also allow for lower effective doses of conventional antifungals, thereby mitigating their significant side effects (e.g., nephrotoxicity of polyenes, hepatotoxicity of azoles). This approach effectively "repurposes" and extends the utility of existing, limited antifungal classes. Synergy often implies that the natural product targets a different pathway or enhances drug uptake/action, creating a multi-target effect that is more difficult for the fungus to overcome. Thus, synergistic combinations represent a practical and immediate strategy to combat antifungal resistance and improve patient outcomes, leveraging the strengths of both natural and synthetic compounds.

3.3. Alkaloids

Alkaloids constitute a diverse group of natural compounds, predominantly found in plants, recognized for a wide array of biological activities, including significant antifungal properties. Sources include *Phellodendron amurense* and *Coptidis rhizoma* for berberine; *Piper nigrum* for piperine; *Fibraurea recisa* for roemerine; *Eschscholzia californica* for hunnemanine and norsanguinarine; and *Fumaria indica* for fuyuziphine.

Key alkaloid compounds with anti-*Candida* activity include berberine hydrochloride, piperine, roemerine, palmatine hydrochloride, hunnemanine, norsanguinarine, fuyuziphine, matrine, and various triterpenoidal alkaloids and β-carboline derivatives.

The antifungal mechanisms of alkaloids often focus on anti-virulence properties:

- **Biofilm Inhibition:** Piperine significantly halts *C. albicans* biofilm formation. Roemerine has also been shown to inhibit biofilm formation.
- **Morphological Transition Inhibition:** Roemerine suppresses the critical yeast-to-hyphae transition in *C. albicans*. Similarly, piperine has been observed to suppress hyphal transition.
- **Membrane Permeability:** Piperine increases cell membrane permeability and disrupts mitochondrial membrane potential. Alkaloids generally contribute to the disruption of cell membrane permeability.
- **Mitochondrial Disruption:** Essential oils, which can contain alkaloids, have been shown to disrupt the electron transport system by rupturing the mitochondrial membrane.
- **Metabolic Interference:** Alkaloids can interfere with fundamental fungal metabolic processes, including nucleic acid and protein synthesis.
- **Synergistic Effects:** Berberine has demonstrated synergistic action with fluconazole against resistant *C. albicans* strains.

The prominent anti-virulence properties of alkaloids, particularly their ability to inhibit biofilm formation and morphological transitions, present a strategic advantage in antifungal

therapy. Traditional antifungals are fungicidal or fungistatic, directly killing or inhibiting growth, which creates strong selective pressure for resistance. Anti-virulence agents, however, disable the pathogen's ability to cause disease (e.g., by preventing adhesion, biofilm formation, or tissue invasion) without necessarily killing it. This "disarming" approach is hypothesized to reduce the evolutionary pressure for resistance, as the fungus is not under existential threat. If a fungus cannot form a biofilm or invade tissues, its clinical impact is drastically reduced, even if it remains viable. This represents an innovative approach to combat resistance. Alkaloids, with their demonstrated anti-virulence capabilities, therefore offer a promising avenue for developing novel antifungal therapies that prioritize mitigating pathogenicity and slowing resistance evolution, rather than solely focusing on direct fungal elimination.

3.4. Coumarins

Coumarins are a class of aromatic oxygen-containing heterocyclic compounds found naturally in various plants. Coumarin (1,2-benzopyrone) itself is a well-known example , and scopoletin is another natural coumarin isolated from plants such as

Mitracarpus frigidus.

The antifungal mechanisms of coumarins primarily involve targeting fundamental cellular processes essential for fungal survival:

- **Apoptosis Induction:** Coumarin induces programmed cell death (apoptosis) in *C. albicans* by triggering a series of apoptotic features, including phosphatidylserine externalization, DNA fragmentation, and nuclear condensation.
- **Mitochondrial Dysfunction:** Coumarin significantly impacts mitochondrial function. It leads to an increase in intracellular Reactive Oxygen Species (ROS) levels, alters mitochondrial membrane potential and morphology, and elevates both cytosolic and mitochondrial Ca2+ levels. This cascade results in the release of cytochrome c from mitochondria to the cytoplasm and subsequent activation of metacaspases, enzymes crucial for executing the apoptotic program. Crucially, mitochondrial Ca2+ influx is a key event in coumarin-induced

  C. albicans apoptosis.

- **Cell Wall and Membrane Damage:** Scopoletin has been shown to interfere with the synthesis of essential fungal cell components and disrupt the cell wall and plasma membrane. Coumarin itself can damage the fungal cell by forming pores on the cell wall, leading to leakage and necrosis of cytoplasmic content.
- **Efflux Pump Inhibition:** Scopoletin has been implicated in the inhibition of efflux pumps, particularly when used in association with fluconazole, suggesting a potential to reverse drug resistance mechanisms.

- **Biofilm Reduction:** Scopoletin has demonstrated the ability to reduce the growth rate of preformed *C. tropicalis* biofilms and inhibit biofilm formation.
- **Ergosterol Content Reduction:** Silver (I)-coumarin complexes exert fungicidal activity by disrupting cytochrome synthesis, which leads to respiration inhibition and a reduction in ergosterol biosynthesis.

Coumarins' ability to induce apoptosis and disrupt mitochondrial function in *Candida* cells signifies a potent mechanism that targets fundamental cellular processes essential for fungal survival. By triggering programmed cell death, coumarin forces the fungal cell to self-destruct. The preceding events—ROS generation, mitochondrial dysfunction, and Ca2+ dysregulation—are critical upstream signals that initiate this process. Mitochondria are the "powerhouses" of the cell; disrupting their function (membrane potential, morphology) starves the cell of energy and generates toxic byproducts (ROS). Calcium dysregulation is a universal stress signal. By hitting these fundamental, interconnected processes, coumarin creates a systemic cellular collapse rather than just inhibiting a single enzyme. This makes it harder for the fungus to develop resistance through a simple bypass or single mutation, suggesting a robust fungicidal effect that is less susceptible to single-gene resistance mutations compared to target-specific inhibitors.

3.5. Saponins

Saponins are a diverse group of natural products, found in over a hundred plant families, where they often serve as an integral part of the plant's defense mechanism. Examples of saponin-rich plants include

Mitracarpus frigidus and

Moringa laurifolia. Key saponin compounds identified with anti-

Candida activity include aginoside, A16, A19, A7, A20, A24, and A25.

Saponins exhibit a sophisticated dual mode of action against *Candida*:

- **Membrane Disruption:** Saponins are known for their ability to form pores in lipid bilayers, which increases cellular permeability and leads to the leakage of essential intracellular components. They are believed to preferentially bind to fungal ergosterol, the primary sterol in fungal cell membranes, over mammalian cholesterol, contributing to their selective toxicity. This disruption makes fungal cells more susceptible to osmotic stress induced by external factors like salt.
- **Biofilm Disruption:** Saponins have demonstrated the ability to disrupt and inhibit *C. albicans* biofilm formation and hyphae development, even at concentrations below their minimal inhibitory concentrations (MICs).

- **Gene Regulation:** A particularly compelling mechanism is the ability of *Moringa laurifolia*-derived saponins to downregulate the expression of key virulence genes in *C. albicans*. These include *SAP3* and *SAP5* (encoding secreted aspartyl proteinases) and *ALS3* (encoding an adhesin-like sequence). This gene-regulatory impact suggests a deeper, more targeted interference with

  Candida's pathogenic machinery.

- **Enhanced Drug Penetration:** Saponins can increase cell permeability, which facilitates the penetration of other photosensitizer compounds for photodynamic inactivation, thereby enhancing their antifungal efficacy.

The discovery that saponins not only directly disrupt fungal membranes and biofilms but also downregulate the expression of key virulence genes (SAPs, ALS3) represents a sophisticated dual mode of action. This gene-regulatory impact suggests a deeper, more targeted interference with *Candida*'s pathogenic machinery, potentially leading to a more profound and sustained anti-virulence effect beyond transient physical disruption. While membrane disruption and biofilm inhibition are direct, immediate physical assaults on the fungal cell and its protective structures, the downregulation of virulence genes means the fungus produces less of the proteins that enable it to invade tissues (SAPs) or adhere to surfaces (ALS3). This is a more fundamental interference with the pathogen's "armamentarium," preventing the construction of new virulence factors. This could lead to a more sustained reduction in virulence and potentially a lower selective pressure for resistance, as the fungus is disarmed rather than killed outright. Saponins therefore offer a compelling therapeutic profile by combining direct fungicidal/anti-biofilm effects with a gene-regulatory mechanism that actively suppresses the production of key virulence factors.

4. Challenges and Future Directions in Natural Product Antifungal Drug Development

Despite the compelling *in vitro* and preclinical *in vivo* evidence supporting the antifungal potential of natural products, their translation into clinically approved therapies faces significant and interconnected challenges. Addressing these hurdles is critical for harnessing the full therapeutic promise of these compounds.

4.1. Limitations: Bioavailability, Standardization, and Toxicity Concerns

The journey from a promising natural compound to a marketable drug is fraught with obstacles.

- **Poor Bioavailability and Aqueous Solubility**
- **Standardization Issues**
- **Toxicity Concerns**
- **"Rediscovery" Problem**

- **Drug Interactions**

These limitations are deeply interconnected, creating a complex bottleneck for clinical translation. Poor standardization directly impacts reproducibility and bioavailability, making robust clinical trials difficult and raising safety concerns. Addressing these challenges requires a holistic approach that integrates advanced analytical techniques, improved formulation strategies, and rigorous regulatory oversight.

Conclusions

The global burden of *Candida* infections continues to escalate, driven by an increasing incidence, high mortality rates, and a concerning shift towards multidrug-resistant non-*albicans Candida* species. The formidable challenge of antifungal resistance is rooted in complex molecular mechanisms, including efflux pump overexpression, target enzyme alterations, and the pervasive protection offered by biofilm formation. These resistance strategies, coupled with *Candida*'s sophisticated array of virulence factors—such as morphological plasticity, adhesion, secreted hydrolytic enzymes, and intricate immune evasion—underscore the urgent need for innovative therapeutic solutions.

Natural products present a compelling and diverse reservoir for the discovery of next-generation antifungal agents. Their inherent chemical diversity and often multi-targeted mechanisms of action offer a strategic advantage over conventional single-target drugs, making it more challenging for fungi to develop resistance. The evidence strongly supports their potential:

- **Multi-Mechanistic Action:** Natural compounds from essential oils, polyphenols, alkaloids, coumarins, saponins, and peptides demonstrate a broad spectrum of antifungal mechanisms, including membrane disruption, cell wall inhibition, apoptosis induction, and interference with vital metabolic pathways.
- **Anti-Virulence Properties:** Crucially, many natural products exhibit potent anti-virulence effects, inhibiting biofilm formation, suppressing morphological transitions (yeast-to-hyphae), interfering with adhesion, and downregulating the production of secreted enzymes. This "disarming" approach can reduce pathogenicity and potentially lower the selective pressure for resistance.
- **Synergistic Potential:** The demonstrated synergistic activity of certain natural products with conventional antifungals is particularly significant, offering a pathway to enhance the efficacy of existing drugs, reduce their toxicity, and potentially overcome resistance in recalcitrant infections.
- **Preclinical Validation:** Extensive *in vitro* studies and promising results from diverse *in vivo* animal models provide strong preclinical validation, bridging the gap between laboratory discovery and clinical application.

Despite this immense potential, the clinical translation of natural products faces substantial hurdles, including poor bioavailability, lack of standardization, potential toxicity, and complex drug-drug interactions. These challenges are interconnected, necessitating a holistic approach to drug development.

To fully harness the antifungal potential of natural products, future efforts must focus on:

- **Advanced Formulation and Delivery:** Developing innovative drug delivery systems (e.g., nanoparticles, liposomes) to improve bioavailability, targeted delivery, and stability.
- **Rigorous Standardization:** Implementing comprehensive quality control measures across the entire production chain to ensure consistent composition, efficacy, and safety.
- **Rational Drug Design:** Employing structural modification and computational approaches to enhance potency, selectivity, and pharmacokinetic profiles, and to develop hybrid molecules.
- **Novel Target Discovery:** Investing in basic research and bioinformatics to identify and validate new fungal-specific targets that can circumvent existing resistance mechanisms.
- **Combination Therapies:** Prioritizing the development of synergistic combinations of natural products with conventional drugs or other natural compounds.
- **Immunomodulation:** Exploring the potential of natural products to enhance host immune responses against *Candida*.

In conclusion, natural products offer a critical and promising avenue for addressing the escalating crisis of *Candida* infections and antifungal resistance. By strategically leveraging their multi-targeted and anti-virulence properties, coupled with advancements in pharmaceutical science and a concerted, multidisciplinary effort, these compounds can pave the way for more effective, less toxic, and more resilient antifungal therapies in the future.

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