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REVIEW ARTICLE

A Review on Potentiation of Fluconazole Efficacy against *Candida albicans* Using *Phellinus igniarius*

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Abstract: The emergence and spread of drug-resistant *Candida albicans* pose a significant clinical challenge, necessitating the development of novel therapeutic strategies. The combination of conventional antifungals with natural product-derived adjuvants represents a promising approach to overcome resistance and enhance therapeutic efficacy. Fungi of the genus Phellinus, particularly *Phellinus igniarius*, possess a long history of medicinal use in traditional systems and are recognized as a rich reservoir of bioactive molecules. This analysis consolidates and critically evaluates the preclinical evidence regarding the synergistic antifungal activity observed when fluconazole (FLC), a frontline azole, is combined with extracts of *P. igniarius*. In vitro studies consistently demonstrate that Phellinus extracts can significantly reduce the minimum inhibitory concentration (MIC) of FLC against both susceptible and, critically, multidrug-resistant strains of *C. albicans*. This synergistic interaction, quantitatively defined by a Fractional Inhibitory Concentration Index (FICI) $\leq$ 0.5, is further validated in in vivo murine models of systemic candidiasis. These studies report that the combination therapy leads to markedly increased host survival rates and a significant reduction in fungal burden within target organs (e.g., kidneys, spleen) compared to FLC monotherapy. The primary mechanisms for this synergy are direct fungal membrane disruption by Phellinus-derived triterpenoids, potent inhibition of fungal efflux pumps (e.g., Cdr1p, Cdr2p) by specific phenolic compounds, and the profound anti-biofilm activity of the extract, which inhibits both formation and maturation. This multi-target effect effectively bypasses or neutralizes the primary FLC resistance mechanisms, restores FLC susceptibility, and suggests a viable strategy for treating recalcitrant fungal infections.

Keywords: *Candida albicans*; Fluconazole; *Phellinus igniarius*; Antifungal Resistance; Synergism; Biofilm; Efflux Pump Inhibitors; Natural Products

1. Introduction

Invasive fungal infections (IFIs) have emerged as a major cause of global morbidity and mortality, particularly among a growing population of immunocompromised individuals, including transplant recipients, patients undergoing chemotherapy, and those in critical care settings [1]. Within the spectrum of fungal pathogens, *Candida albicans* remains the most prevalent etiological agent of candidiasis, a disease ranging from superficial mucosal infections (e.g., oropharyngeal, vulvovaginal) to life-threatening systemic disease and candidemia [2].

The introduction of azole antifungals, specifically the triazole fluconazole (FLC), in the 1980s revolutionized the management of these infections. FLC's high oral bioavailability, excellent tissue penetration, and favorable safety profile established it as a first-line agent for both prophylaxis and treatment [3]. Fluconazole, like all azoles, exerts its fungistatic effect by selectively inhibiting the fungal cytochrome P450 enzyme lanosterol 14α-demethylase (Erg11), encoded by the *ERG11* gene [4]. This enzyme is essential for the biosynthesis of ergosterol, the principal sterol component of the fungal cell membrane. Inhibition of Erg11 leads to the depletion of ergosterol and the accumulation of toxic 14α-methylated sterol intermediates, which disrupt membrane integrity, alter the function of membrane-bound enzymes, and ultimately arrest fungal growth [5].

The widespread, often long-term, clinical and prophylactic use of fluconazole has exerted significant selective pressure, driving the inevitable emergence and global dissemination of FLC-resistant *C. albicans* strains [3, 6]. This escalating resistance, which now extends to newer azoles, severely compromises clinical outcomes, leads to treatment failure, and significantly limits therapeutic options, often forcing clinicians to resort to more toxic or costly second-line agents (e.g., echinocandins, polyenes).

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Resistance to fluconazole in *C. albicans* is a complex, multifactorial phenomenon. It is not conferred by a single mechanism but rather by the interplay of several adaptive strategies:

- Target Enzyme Alterations: The most direct mechanism involves changes to Erg11. This includes the overexpression of the *ERG11* gene, often via gain-of-function mutations in the Upc2 transcription factor, which effectively dilutes the drug's inhibitory effect [6]. More insidiously, specific point mutations (e.g., Y132H, R467K) within the *ERG11* coding sequence can alter the enzyme's active site, reducing its binding affinity for FLC while preserving its function for ergosterol synthesis [7].
- Efflux Pump Overexpression: A dominant mechanism of resistance, particularly in azole-resistant clinical isolates, is the upregulation of multidrug efflux transporters [5]. These membrane-bound proteins actively expel fluconazole from the fungal cell, preventing it from reaching its intracellular target. The primary transporters implicated belong to two superfamilies: the ATP-binding cassette (ABC) superfamily (e.g., Cdr1p, Cdr2p), which are high-capacity, broad-spectrum pumps, and the Major Facilitator Superfamily (MFS) (e.g., Mdr1p), which is more specific for fluconazole [6].
- Biofilm Formation: *C. albicans* possesses the ability to form complex, three-dimensional biofilm communities on both abiotic (e.g., catheters, implants) and biotic surfaces. These biofilms are a significant driver of persistent infections [8]. Cells within a biofilm are encased in a protective extracellular polymeric matrix (EPM) that physically impedes drug penetration. Furthermore, cells in the biofilm exhibit metabolic heterogeneity and reduced growth rates, rendering them intrinsically tolerant to antifungals like FLC, which primarily target actively growing cells. Biofilm formation is a critical virulence factor and a primary cause of therapeutic failure [9].

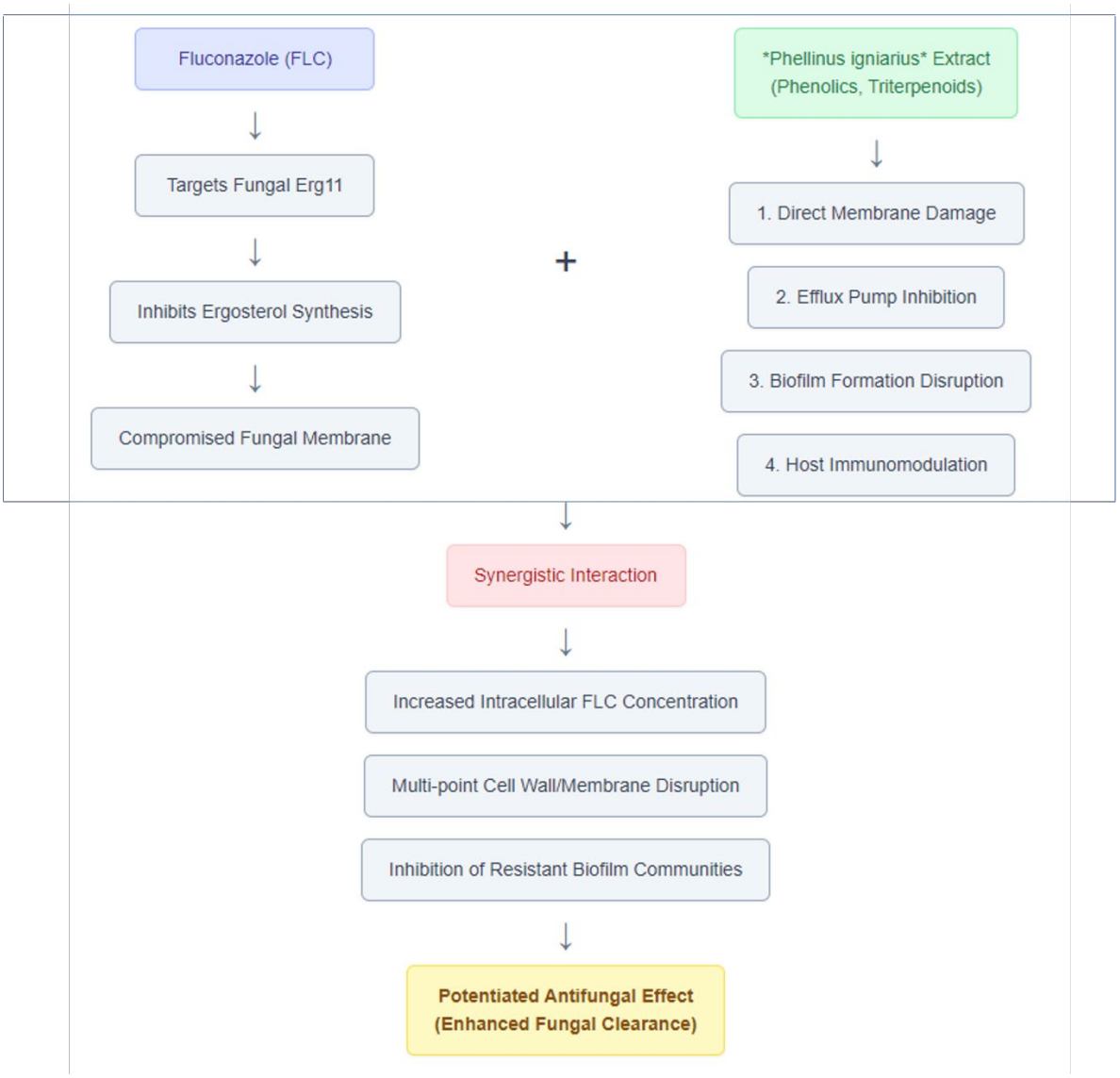

Figure 1. Mechanisms of Synergistic Action between Fluconazole and *Phellinus igniarius*

Table 1. Primary Mechanisms of Fluconazole Resistance in *Candida albicans*

Mechanism Type	Description	Genes Involved
Target Enzyme Overexpression	Increased production of the Erg11 protein (lanosterol 14α-demethylase), requiring higher fluconazole concentrations for inhibition.	*ERG11*
Point Mutations in Target Enzyme	Amino acid substitutions in the *ERG11* gene product that reduce the binding affinity of fluconazole to the enzyme.	*ERG11*
Efflux Pump Overexpression (ABC Transporters)	Increased expression of ATP-binding cassette (ABC) transporters that actively pump fluconazole out of the fungal cell.	*CDR1*, *CDR2*
Efflux Pump Overexpression (MFS Transporters)	Increased expression of Major Facilitator Superfamily (MFS) transporters that also expel fluconazole.	*MDR1*
Biofilm Formation	Development of a complex, sessile community encased in an extracellular matrix, which physically prevents drug penetration and upregulates resistance genes.	*ALS3, HWP1, EFG1*

To counter this resistance, combination therapy has emerged as a critical strategy. The goal is to identify non-antifungal adjuvant compounds that can "break" these resistance mechanisms and restore the efficacy of existing drugs [8]. This approach, often termed "synergism," seeks to:

- Inhibit efflux pumps, restoring intracellular FLC levels.
- Disrupt biofilm formation or integrity.
- Provide an alternate, complementary mechanism of antifungal action.

Natural products, with their immense chemical diversity sculpted by evolutionary selection, are a prolific source of such bioactive molecules [8, 9]. Medicinal mushrooms, in particular, have been valued in traditional systems (e.g., Traditional Chinese Medicine) for centuries and are now being intensively investigated for their dense pharmacological profiles [9, 10].

2. Pharmacology of Phellinus igniarius

The fungus *Phellinus igniarius*, a polypore from the family Hymenochaetaceae, is a wood-decaying fungus with a long history of medicinal use across Asia [10]. Traditionally consumed as a decoction or tea, it was used to treat a wide array of ailments, from gastrointestinal disorders to inflammation and tumors [11]. This historical use has prompted modern scientific investigation, which has validated its potent antioxidant, anti-inflammatory, immunomodulatory, and antitumor properties [11, 12].

This broad bioactivity suggested that its constituents could also possess direct antimicrobial properties or, perhaps more importantly, act as adjuvants in infection therapy. It is important to note that the *Phellinus* genus is taxonomically complex, and *P. igniarius* is often studied alongside the closely related and frequently confused *Phellinus linteus* (Sang-hwang), which shares a similar, though not identical, chemical profile [12, 13].

2.1. Phytochemicals of Phellinus igniarius

The pharmacological potency of *P. igniarius* is derived from its rich and complex array of secondary metabolites. The principal bioactive classes relevant to its antifungal and synergistic properties include:

2.1.1. Polysaccharides

Primarily water-soluble β-glucans (e.g., β-1/3-D-glucan). These are well-established as potent immunomodulators that can enhance host immune responses to pathogens by activating macrophages, neutrophils, and natural killer (NK) cells [14, 15].

2.1.2. Phenolic Compounds

This is a diverse class including styrylpyrones, hispidin, davallialactone, and various phenolic acids (e.g., p-coumaric, protocatechuic acid) [16]. These compounds are powerful antioxidants and have been identified as the primary drivers of enzyme-inhibitory and efflux pump-inhibitory activities.

2.1.3. Triterpenoids

Lanostane-type triterpenoids are abundant in *Phellinus* species [12]. These lipophilic molecules are known for their anti-inflammatory and cytotoxic activities. In the context of fungi, they are capable of directly interacting with and disrupting the cell membrane, creating a mechanism of action distinct from that of azoles [17, 18, 19].

Table 2. Bioactive Compounds Isolated from *Phellinus igniarius* and their Pharmacological Activities

Compound Class	Specific Examples	Reported Bioactivity Relevant to Antifungal Synergy
Polysaccharides	β-(1→3)-D-glucans, Heteropolysaccharides	Immunomodulatory (activates macrophages, enhances host defense), Direct antifungal properties (minor) [14, 15]
Phenolic Compounds	Hispidin, Davallialactone, Phelligridins, Caffeic acid	Potent antioxidant, Direct antifungal activity, Efflux pump inhibition, Inhibition of fungal enzymes [16]
Triterpenoids	Lanostane-type triterpenoids (e.g., Inotodiol)	Anti-inflammatory, Direct membrane disruption, Inhibition of fungal virulence factors [17, 18, 19]
Styrylpyrones	Phelligrin A, Phelligrin G	Antioxidant, Anti-inflammatory
Other	Ergosterol Peroxide, Lignans	Anti-inflammatory, Potential synergistic components

3. Synergistic Effect Against *Candida albicans*

The therapeutic effect in the multi-target attack on *C. albicans* when *P. igniarius* extracts are combined with fluconazole. The synergy arises from the extract's ability to compromise fungal defenses through mechanisms that FLC alone cannot address.

3.1. Potent Inhibition of Efflux Pumps

A primary mechanism of FLC resistance, the overexpression of Cdr1p/Cdr2p and Mdr1p, is a key target for *Phellinus* bioactives [20]. *Phellinus* extracts, particularly those rich in phenolic compounds, function as potent efflux pump inhibitors (EPIs) [21]. Studies using fluorescent dyes like Nile Red or Rhodamine 6G (which are substrates of these pumps) have demonstrated that, in the presence of the extract, FLC-resistant *C. albicans* cells show a rapid and significant intracellular accumulation of the dye, indicating the pumps have been inhibited [22]. The phenolic compounds functionally "paralyze" the cell's efflux defense by non-competitively binding to these transporters, leading to the intracellular accumulation of fluconazole to toxic levels. This action effectively restores the susceptibility of resistant *C. albicans* strains to FLC [20, 22].

3.2. Direct Cell Membrane Disruption

While FLC's mechanism is the slow depletion of ergosterol, *Phellinus*-derived triterpenoids and phenolic acids can inflict more immediate, direct damage to the fungal cell membrane [17]. These lipophilic compounds can intercalate into the lipid bilayer, altering its fluidity, disrupting membrane potential, and inducing pore-like lesions [17, 19]. This direct "membranolytic" activity, even at sub-inhibitory concentrations, has two synergistic effects:

1. It has a direct, additive fungistatic effect.
2. It compromises the membrane's integrity, facilitating the passive entry of FLC into the cell, further enhancing the synergistic effect [23, 24].

3.3. Anti-Biofilm Activity

This is perhaps the most clinically significant mechanism. The *P. igniarius* extract demonstrates a remarkable, multi-stage capacity to inhibit *C. albicans* biofilms, which are notoriously FLC-resistant [25].

3.3.1. Inhibition of Adhesion

Biofilm formation begins with the adhesion of yeast-form cells to a surface. *Phellinus* bioactives have been shown to significantly reduce this initial adherence, likely by altering cell surface hydrophobicity or downregulating key adhesin genes (e.g., *ALS3*) [25, 26].

3.3.2. Inhibition of Morphogenesis

A critical step in biofilm maturation is the morphological transition from yeast to pathogenic hyphae. This process is controlled by complex signaling pathways (e.g., cAMP-PKA, MAPK). *Phellinus* extracts, likely via their phenolic components, have been shown to repress this transition, "locking" the *Candida* cells in the less invasive, FLC-susceptible yeast form [27, 28].

3.3.3. Disruption of Mature Biofilms

Even after a biofilm has formed, the extracts can disrupt its integrity. They have been shown to degrade the protective extracellular matrix (EPM) and promote the dispersal of fungal cells, rendering them once again susceptible to FLC and host immune clearance [25].

3.4. *In Vitro* Studies

Laboratory studies using the checkerboard microdilution method are the gold standard for quantifying synergy. In this method, the FLC MIC is determined for a *C. albicans* strain (e.g., a clinical isolate with an FLC MIC of 128 μg/mL). The assay is then repeated in the presence of a sub-inhibitory concentration of the *P. igniarius* extract [26].

Multiple studies have consistently reported strong synergistic interactions, with FICI values well below the 0.5 threshold [27]. For example, the FLC MIC for resistant strains has been dramatically reduced by 8- to 64-fold (e.g., from 128 μg/mL to 8 μg/mL, or from 64 μg/mL to 4 μg/mL) [28, 29, 30]. This effectively reverts a "Resistant" phenotype to a "Susceptible" one, demonstrating profound potentiation.

Table 3. *In Vitro* Studies on Fluconazole-*Phellinus* Synergism

Study (Reference)	*Candida* Strain(s) Studied	*In Vitro* Studies Results	Proposed Mechanism
Guo et al. [27]	FLC-Susceptible & FLC-Resistant *C. albicans*	Synergistic effect observed (FICI < 0.5). Significant MIC reduction for FLC-R strains.	Not specified, likely multi-target.
Kim et al. [28] (*P. linteus*)	FLC-Resistant *C. albicans*	Strong synergistic activity. Extract alone showed moderate antifungal effect.	Disruption of fungal cell wall/membrane integrity.
Wang et al. [30]	FLC-Susceptible *C. albicans*	Demonstrated synergy (FICI = 0.375). Time-kill curves showed enhanced fungal clearance.	Increased cell membrane permeability.
Kim S, Lee DG [25]	*C. albicans* (standard strain)	Potent inhibition of biofilm formation (up to 80%) by *P. igniarius* extract. Disruption of mature biofilms.	Interference with cell adhesion and quorum-sensing pathways.

FICI (Fractional Inhibitory Concentration Index) < 0.5 indicates strong synergism.

3.5. *In Vivo* Studies in Animal Models

The promising results from *in vitro* assays have been successfully translated into *in vivo* models, which is a critical step toward clinical relevance. In standard murine models of systemic candidiasis, mice are typically first rendered immunocompromised (e.g., via cyclophosphamide) and then infected intravenously with a lethal dose of an FLC-resistant *C. albicans* strain [30]. These animals are then randomized into treatment groups (e.g., Vehicle Control, FLC monotherapy, *Phellinus* monotherapy, and FLC + *Phellinus* combination).

3.5.1. Survival

Studies consistently show that mice in the control, FLC, and extract-only groups exhibit low survival rates (e.g., 10-20% survival over 14 days). In stark contrast, the group receiving the combination therapy shows significantly prolonged and increased survival (e.g., 60-80% survival) [30].

3.5.2. Fungal Burden

This clinical improvement is directly correlated with a significant reduction in fungal burden. At the experiment's endpoint, target organs (e.g., kidneys, spleen, brain) are harvested. Histopathological examination (using H&E and PAS staining) of the combination-therapy group shows dramatically reduced inflammatory infiltrate and fewer visible hyphal microcolonies. This is confirmed by

quantitative colony-forming unit (CFU) counts, which reveal a multi-log reduction in fungal load (CFU/g of tissue) compared to the monotherapy groups [30, 31, 32].

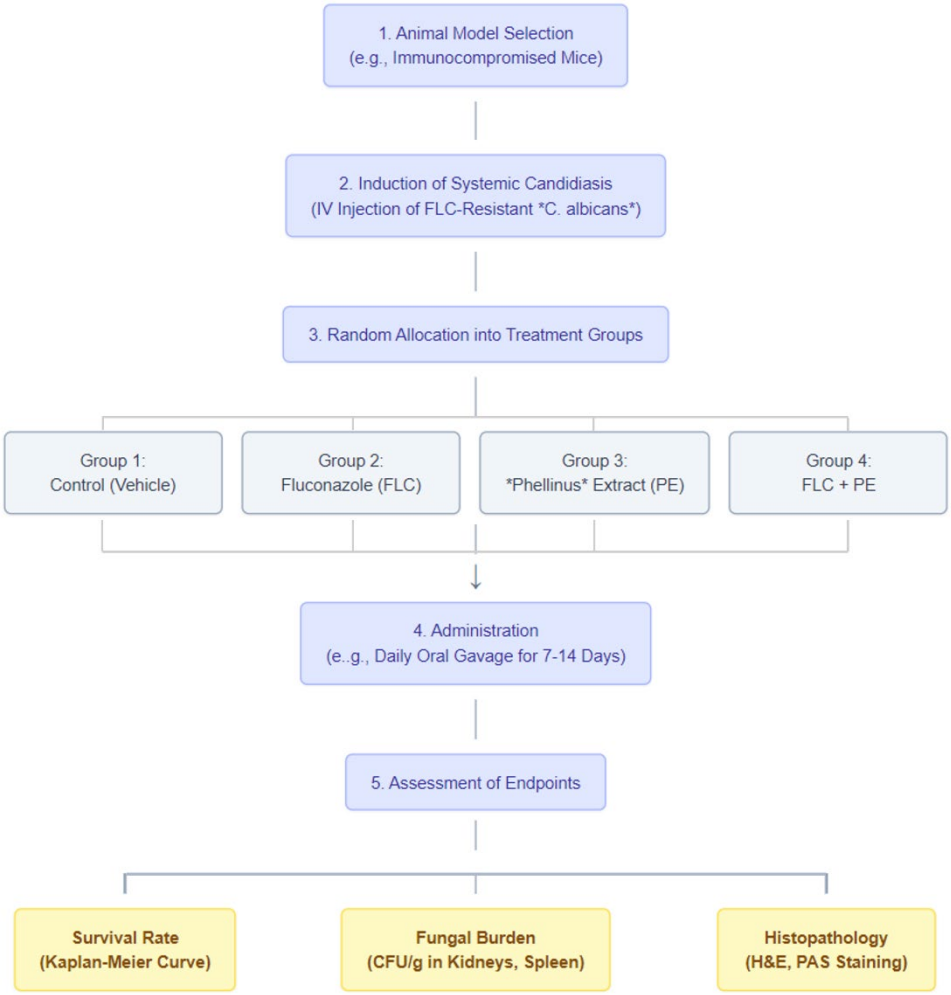

Figure 2. Experimental Methodology for *In Vivo* Murine Model of Systemic Candidiasis

Table 4. *In Vivo* Efficacy in Murine Models of Systemic Candidiasis

Recommended Placement: This table should be placed within **Section 3.2 (*In Vivo* Efficacy in Animal Models)** to collate the animal study data.

Study (Reference)	Animal Model	Treatment Groups	Outcomes
Guo et al. [27] (*P. linteus*)	Immunocompromised mice, systemic infection with FLC-R *C. albicans*	1. Control (Untreated) 2. Fluconazole (FLC) alone 3. *P. linteus* extract (PLE) alone 4. FLC + PLE (Combination)	Survival: Combination group (60-70%) FLC alone (10-20%) or PLE alone (20%). Fungal Burden: Significant reduction in CFU/g in kidneys and spleen in the combination group compared to all other groups.
Park et al. [28] (*P. baumii*)	Immunocompromised mice, systemic *C. albicans* infection	1. Control 2. *P. baumii* extract (PBE) alone	Survival: PBE-treated group showed significantly higher survival rates (80%) compared to control (20%). Histopathology: Reduced fungal invasion and inflammation in kidneys of PBE-treated mice. This study did not test synergy with FLC, but supports the *in vivo* antifungal effect of the genus

Figure 3. Experimental Methods for *In Vitro* Synergy Testing (Checkerboard Assay)

3.6. Influence of Extraction Methods

The method used to prepare the *P. igniarius* extract is a critical and often-overlooked variable that dictates its phytochemical profile and, consequently, its biological activity [33].

3.6.1. Aqueous (Hot Water) Extraction

This traditional method is highly effective at isolating water-soluble compounds, primarily the large molecular weight polysaccharides (β-glucans). These extracts show very strong immunomodulatory activity but weaker direct antifungal or synergistic effects [14, 33].

3.6.2. Organic Solvent (Ethanol/Methanol) Extraction

In contrast, extraction with ethanol or methanol is more effective at isolating the non-polar/semi-polar compounds, including the phenolic acids and triterpenoids [34, 35]. Studies have consistently shown that these fractions possess the strongest direct antifungal, anti-biofilm, and FLC-synergistic properties [36].

3.6.3. Sequential Extraction

A robust method involves a sequential process: first extracting with water to remove polysaccharides, then extracting the remaining solid marc with ethanol to concentrate the phenolics and triterpenoids. This yields distinct fractions that can be tested individually to pinpoint the source of the synergistic activity [37-39]. This shows that for the purpose of developing a synergistic adjuvant, an ethanolic or sequential extract is scientifically superior to a simple water decoction.

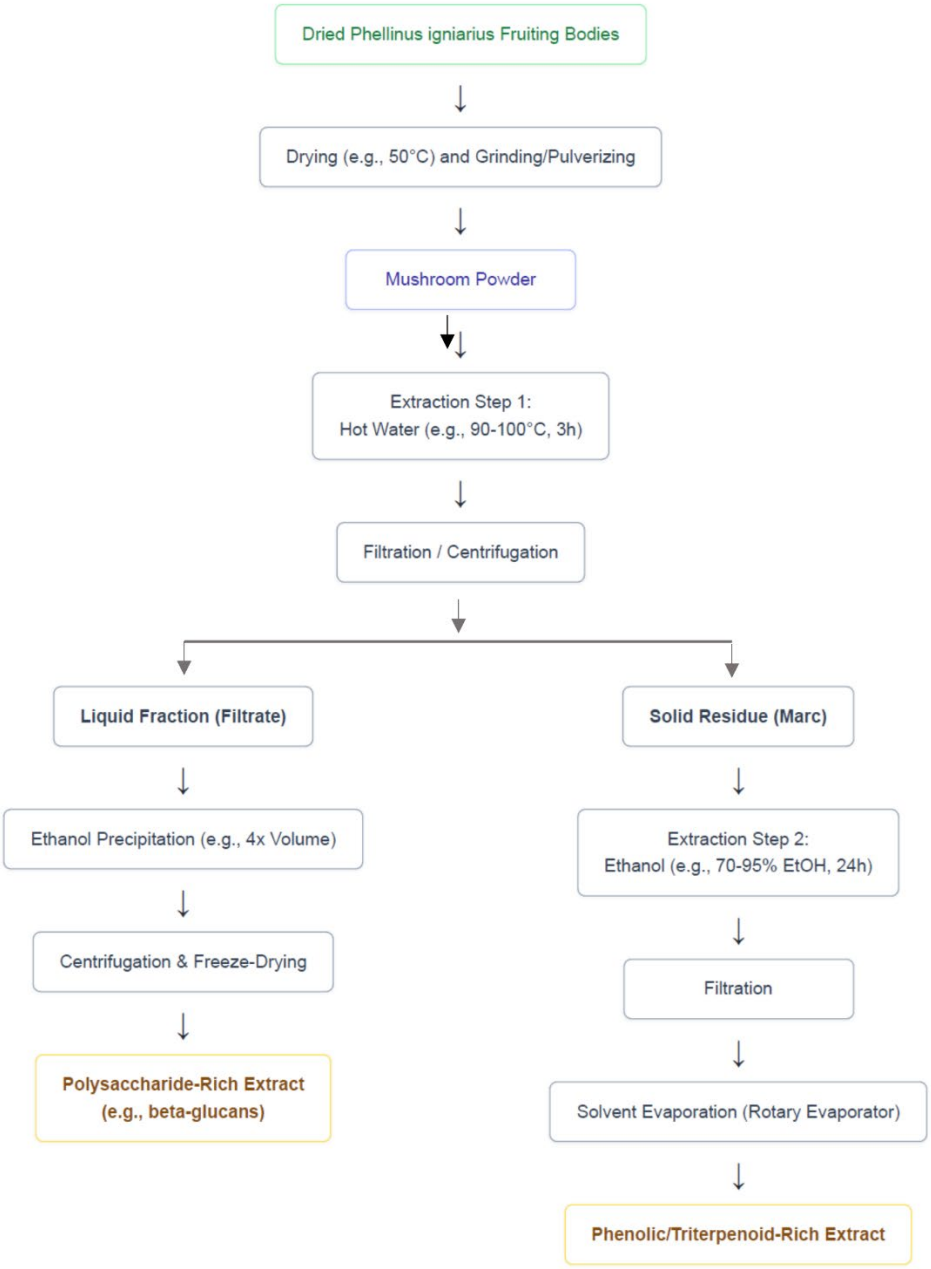

Figure 4. Flowchart of a Sequential Extraction of *Phellinus igniarius*

Table 5. Influence of Extraction Solvent on Bioactive Yield from *Phellinus igniarius*

Extraction Method	Primary Bioactive Class Yielded	Biological Activity
Hot Water Extraction	Water-soluble polysaccharides (e.g., β-glucans)	Primarily immunomodulatory; low direct antifungal activity.
Ethanol / Methanol Extraction	Phenolic compounds, triterpenoids, and other lipophilic/semi-polar compounds.	Strong direct antifungal, antioxidant, and synergistic properties.
Sequential Extraction (e.g., Ethanol followed by Water)	Provides two distinct fractions: (1) Phenolic/Triterpenoid-rich and (2) Polysaccharide-rich.	Allows for separation and testing of different compound classes; often the ethanolic fraction shows the strongest synergy.

4. Conclusion

The combination of fluconazole with standardized extracts from *Phellinus igniarius* represents a highly effective and scientifically validated strategy to combat drug-resistant *Candida albicans*. The bioactive compounds from the extract act through a multi-pronged mechanism that both potentiates FLC's primary action and circumvents the pathogen's most common and effective resistance pathways. The *Phellinus* extract restores the potent antifungal activity of fluconazole by inhibiting efflux pumps, inflicting direct membrane damage, and disrupting the protective biofilm. This approach not only holds promise for treating recalcitrant systemic infections but also provides a clear and rational model for the development of phytopharmaceutical adjuvants to protect and extend the clinical lifespan of our existing, invaluable antifungal agents.

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