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Review

# Antifungal effects of phytocompounds on *Candida* species alone and in combination with fluconazole

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ABSTRACT

Invasive fungal infections caused by *Candida* spp. remain the most predominant nosocomial fungal infections. Owing to the increased use of antifungal agents, resistance of *Candida* spp. to antimycotics has emerged frequently, especially to fluconazole (FLC). To cope with this issue, new efforts have been dedicated to discovering novel antimycotics or new agents that can enhance the susceptibility of *Candida* spp. to existing antimycotics. The secondary metabolites of plants represent a large library of compounds that are important sources for new drugs or compounds suitable for further modification. Research on the anti-*Candida* activities of phytocompounds has been carried out in recent years and the results showed that a series of phytocompounds have anti-*Candida* properties, such as phenylpropanoids, flavonoids, terpenoids and alkaloids. Among these phytocompounds, some displayed potent antifungal activity, with minimum inhibitory concentrations (MICs) of ≤8 μg/mL, and several compounds were even more effective against drug-resistant *Candida* spp. than FLC or itraconazole (e.g. honokiol, magnolol and shikonin). Interestingly, quite a few phytocompounds not only displayed anti-*Candida* activity alone but also synergised with FLC against *Candida* spp., even leading to a reversal of FLC resistance. This review focuses on summarising the anti-*Candida* activities of phytocompounds as well as the interactions of phytocompounds with FLC. In addition, we briefly overview the synergistic mechanisms and present the structure of the antimycotic phytocompounds. Hopefully, this analysis will provide insight into antifungal agent discovery and new approaches against antifungal drug resistance.



## 1. Introduction

The incidence of invasive fungal infections caused by *Candida* spp. has increased continuously in recent decades, especially in populations of immunocompromised patients or individuals hospitalised with serious underlying diseases [1]. *Candida* is a yeast-like, eukaryotic, diploid, sexual fungus consisting of ca. 150 species, of which more than 17 species are reported to cause candidiasis in humans [2]. *Candida albicans* remains the most prevalent species worldwide, whilst the prevalence of infections caused by non-*albicans Candida* spp. such as *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei* is increasing [3–5]. These yeasts not only cause superficial fungal infections but also systemic fungal infections such as candidaemia and biofilm-associated infections [6–8]. Candidiasis is usually treated with synthetic antifungal agents such as azoles, polyenes and echinocandins; however these antifungal drugs are limited and some of them cause serious side effects to host tissues [7]. Moreover, on pace with the increasing frequency of candidiasis, there has been a sharp increase in the use of antifungal agents, especially fluconazole (FLC), owing to its efficacy and low toxicity, which in turn induces the development of drug resistance [9]. Therefore, it is of great importance to search for novel compounds that have high anticandidal activity or that can enhance the susceptibility of *Candida* spp. to existing antifungal agents. Specifically, research on enhancing the susceptibility of *Candida* spp. to FLC has attracted considerable attention.

Plants, especially higher plants with ethnopharmacological uses, have been utilised extensively as crude material or pure compounds for treating and preventing human diseases for many centuries, and these historical experiences with plants as therapeutic tools have helped to introduce pure phytocompounds to modern medicine [10,11]. Phytocompounds play an important role in drug discovery by serving as compounds of interest in their natural form or as templates for synthetic modification [12,13]. Many studies

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both of natural phytocompounds and their chemically synthesised derivatives have demonstrated antifungal activities against *Candida* spp. To our knowledge, although there have been some reviews regarding antifungal phytocompounds prior to 2010 [14–18], few reports have summarised the antifungal phytocompounds that were reported after 2010 and the interactions of these phytocompounds with FLC. Therefore, this review primarily focuses on the anti-*Candida* activities of natural phytocompounds reported after 2010, especially those with minimum inhibitory concentrations (MICs) of ≤32 μg/mL (Table 1), as well as the interactions of these phytocompounds with FLC (Table 2). In addition, the structures of these phytocompounds are summarised according to their category (Figs 1–7), and the names of phytocompounds with MIC values ≤8 μg/mL are marked with *.

## 2. Antifungal effects of phytocompounds

Anticandidal activity has been revealed in various classes of phytocompounds. This review presents the antifungal effects of phytocompounds according to their classification.

### 2.1. Phenylpropanoids

Phenylpropanoids (Fig. 1) refer to natural plant ingredients composed of a benzene ring with three carbon units that are normally categorised into coumarins, phenylpropanoic acids and lignans. Natural phenylpropanoids are currently being studied for their anti-*Candida* activities.

A known coumarin (scopoletin) and two phenylpropanoic acids (salicylaldehyde and anisylalcohol) showed moderate antifungal activity against *C. albicans*, with MICs of 25, 31 and 31 μg/mL, respectively [19,20]. Chlorogenic acid, one of the most abundant polyphenols in the human diet, is formed by caffeic and quinic acids; these three compounds displayed MICs of 8 μg/mL against *C. albicans* and 16 μg/mL against *C. parapsilosis*, whilst chlorogenic acid was found to be effective in treating antiseptic arthritis caused by *C. albicans* in an early study [21,92]. Six phenylpropanoic acids (cinnamaldehyde, coniferyl aldehyde, sinapaldehyde, eugenol, methyleugenol and estragole) are all important ingredients in plants and showed slight efficacy against FLC-susceptible ($FLC^S$) and FLC-resistant ($FLC^R$) *Candida* isolates, with MICs exceeding 100 μg/mL in vitro [93–97]. In addition, cinnamaldehyde, coniferyl aldehyde and sinapaldehyde could act by changing the ultrastructure of *C. albicans* in a dose-dependent manner, and eugenol exerted in vivo antifungal activity against *C. albicans* isolates and antibiofilm activity against *C. albicans* biofilms [98–101].

Glochidioboside, a naturally occurring neolignan glucoside, possessed an inhibitory effect against *C. albicans* and *C. parapsilosis* at an MIC of 3.3–6.5 μg/mL [22]. Two neolignan compounds, honokiol and magnolol from *Magnolia obovata*, were both more active against *C. albicans* than itraconazole (ITC), with MICs of 25 μg/mL. These compounds prolonged the survival of nematodes infected with *C. albicans*, indicating their promise for the treatment of *Candida* infections [23,102]. Styraxjaponoside C, a lignan glucoside from *Styrax* spp., exhibited activity against *C. albicans* in an energy-independent manner and demonstrated an MIC of 40 μg/mL [103].

### 2.2. Quinones

Quinones (Fig. 2) are a type of aromatic organic compound containing aromatic rings with two ketone substitutions. There are a few reports regarding their anti-*Candida* activities.

Purpurin, a natural red anthraquinone pigment, significantly inhibited the growth of *Candida* spp., with an MIC range of 1.28–5.12 μg/mL [24]. Purpurin also instigated in vitro effects on biofilm formation of *C. albicans* and *Candida dubliniensis* [24,104,105]. Aloe-emodin and its acetate from *Siraitia grosvenorii* both showed an MIC of 6.1 μg/mL against *C. albicans* [25]. Shikonin, the major constituent of *Lithospermum erythrorhizon*, possessed significant antifungal activity against *C. albicans*, with an $MIC_{80}$ range of 2–8 μg/mL. More importantly, for some $FLC^R$ *C. albicans*, shikonin was more effective than FLC, with an $MIC_{80}$ that was >16 times lower than that of FLC, signifying that shikonin may be a promising candidate as an antifungal agent for the treatment for candidosis [26]. Menadione generally maintains healthy blood clotting, but naturally-occurring menadione also exhibited potent antifungal activity against a $FLC^R$ *C. albicans* strain, with an MIC of 15.6 μg/mL [27]. Hypericin, from the genus *Hypericum*, is endowed with species-dependent antifungal potential against *C. albicans*, *C. parapsilosis* and *C. krusei* when used as a photodynamic therapy drug, and it was effective in the killing of *C. albicans* strains in a resistance-independent manner [106,107].

### 2.3. Flavonoids

Flavonoids (Fig. 3) refer to a series of compounds that have a 2-phenyl chromone as a basic parent nucleus and fall into diverse classes, such as flavones, flavonols, flavanones and biflavones. Recent studies indicated that flavonoids not only had antibacterial and antiviral activity but also had antifungal effects in vitro and in vivo.

#### 2.3.1. Flavones and flavonols

As an important branch of flavonoids, the flavones and flavonols are distributed widely in the plant kingdom. Salazar-Aranda et al. evaluated the in vitro anti-*Candida* activities of baicalein and other flavonols and found that baicalein and myricetin produced obstructive effects on *Candida* spp., with MICs of 1.9–21 μg/mL and 3.9–64 μg/mL, respectively, whilst other compounds were only effective against *C. glabrata*, with MICs of <32 μg/mL (Table 1) [28]. Baicalein also had weak inhibitory effects against amphotericin B-resistant ($AmB^R$) *C. krusei* and *C. parapsilosis* isolates, but predominantly inhibited innate $FLC^R$ *C. krusei* isolates [29,108]. The in vitro antibiofilm activity of baicalein was also observed for *C. albicans*, $AmB^R$ *C. krusei* and *C. parapsilosis* isolates [108,109]. Two dietary flavonoids (isoquercitrin and quercetin) exhibited species-independent effects against *C. albicans* and *C. parapsilosis*, with MICs of 2.5 μg/mL and 8 μg/mL, respectively [21,30], whereas other data suggested that the MIC of quercetin against *C. albicans* was 20 μg/mL [31]. Kaempferol from *Commiphora pedunculata* demonstrated MICs of 6.25 μg/mL against *C. krusei* and 12.5 μg/mL against *C. albicans*, whilst its dihydride had a more considerable MIC of 6.25 μg/mL against both strains [32].

Derrone and licoflavone C from *Retama raetam* had antifungal activities against *Candida* spp., with MICs of 7.81 μg/mL and 15.62 μg/mL, respectively [33]. Quercetin-3-*O*-rutinosides, also named rutin, had therapeutic effects on *Candida* arthritis and showed similar MICs to five other plant-derived flavonoids (16 μg/mL against *C. albicans* and 32 μg/mL against $FLC^R$ *C. krusei*) [34,110]. Papyriflavonol A from *Broussonetia papyrifera* proved to have antifungal activity against *C. albicans*, with an MIC of 25 μg/mL, and caused significant morphological alterations [35].

#### 2.3.2. Flavanones, biflavones and other flavonoids

Pinocembrin and alpinetin from Combretaceae showed inhibitory effects against *C. albicans*, with MICs of 6.25 μg/mL and 25 μg/mL, respectively [36]. Licochalcone A and glabridin from radix glycyrrhizae were reported to inhibit the growth, biofilm formation and yeast–hyphal transition of *C. albicans*, and an early study demonstrated that glabridin was active against *Candida* spp., especially $AmB^R$ *C. albicans* mutants [37,111]. Genistein, a natural isoflavone present in soybeans, showed an MIC of 8 μg/mL both against *C. albicans* and *C. parapsilosis*, whilst the MICs of silibinin