Review

Review of flavonoids: A diverse group of natural compounds with anti-
Candida albicans activity in vitro

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A B S T R A C T

Flavonoids are a subdivision of polyphenols, a versatile class of natural compounds that represent secondary metabolites from higher plants and are abundant in human diet. Various protective effects of flavonoids have been reported, including antimicrobial and antifungal activities. Due to the nature of oral candidiasis and the increased use of antifungal agents, several drug-resistant strains have emerged making it impractical to rely on one standard therapeutic regime. The aim of this review is to summarize the antifungal activity of some examples of the major subclasses of flavonoids in pure extract forms against C. albicans in vitro, as reported in literature over the past 10 years (2004–2015). In addition, this review outlines the potential mechanism of actions of flavonoids studied in vitro, which may contribute to a better understanding of flavonoids as multi-targets agents in the treatment and/or prevention of oral candidiasis in clinical settings.

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Abbreviations: IC50, 50% inhibitory concentration; MIC, minimum inhibitory concentration; MCF50, 50% minimum inhibitory concentration; SAR, structure activity relationship; MABA, microplate alamar blue assay.

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1. Introduction

Oral candidiasis (OC) is one of the most common fungal infections affecting the oral cavity (Das, Nightingale, Patel, & Jumaa, 2011). Candida albicans is a prevalent opportunistic human fungal pathogen that is often implicated in OC and is the most common isolated Candida specie in clinical cases of invasive fungal infections (Liu et al., 2014). C. albicans lives commensally in the gut, oral pharyngeal, genito-urinary tract and skin (Prieto, Correia, Pla,
However, pathogenicity of C. albicans and subsequent candidiasis can occur under immunocompromised conditions (Lalla, Patton, & Dongari-Bagtzoglou, 2013). For instance, the incidence of at least one episode of oral candidiasis in HIV patients is estimated to be 80–95% (Borg-von Zepelin et al., 1999). Furthermore, chemotherapy, certain medications, such as steroids and multiple antibiotic treatments, along with the use of removable dentures may predispose to OC infections (Darwazeh, Hammad, & Al-Jamaei, 2010). As a consequence of oral fungal infections, patients may have dysphagia, weight loss, or disseminated candidiasis. The disseminated forms of the disease can be life-threatening with mortality rates of 35–60% among immunocompromised, cancer patients, and those exposed to multiple treatments, such as broad spectrum antibiotics, chemotherapy, immunosuppressive therapy, and anti-retroviral therapy (Eggi-mann, Que, Revelly, & Pagani, 2015; Gillies et al., 2015; Tang, Liu, Lin, & Lai, 2014).

Despite the availability of broad spectrum triazoles as conventional medical therapies, the incidence of invasive candidiasis continue to increase due to antifungal resistance and the emergence of non-albicans strains of Candida, such as Candida glabrata. The azole fluconazole is currently considered the first-line of drugs that is effective against most Candida species (Lalla et al., 2013; Serpa et al., 2012). However, certain Candida species, such as C. glabrata, C. albicans, C. tropicalis, and C. parapsilosis were found to have different degrees of susceptibility and were reported to have fluconazole resistance (Sanguinetti, Posteraro, & Lass-Florl, 2015). These factors present an urgent need to evaluate novel compounds with antifungal activity. Natural compounds as sources for anti-Candida therapeutics from botanical sources have gained attention in the past decade (2004–2015) mainly because they display structural diversity and uniqueness in functional modes of action, which renders them as attractive candidates to counteract the emergence of Candida drug resistances (Kamba & Hassan, 2010; Toure, Bahi, Ouattara, Djama, & Coulibaly, 2011).

![Fig. 1. Search strategy and papers selection flowchart.](image-url)
Flavonoids are a major class of natural compounds known as polyphenols, which are secondary metabolites naturally occurring in plants and found largely in foods and beverages, such as fruits, vegetables, cereals, tea, coffee, and red wine (Taveira et al., 2010). Epidemiological and some clinical studies have reported the majority of polyphenols to exhibit anti-oxidant and antimicrobial activities including antifungal, anti-viral, and anti-bacterial effects (Yigit, Yigit, & Mavi, 2009).

The aim of this review is to investigate the antifungal activities of flavonoids in vitro against Candida albicans, the most prevalent pathogen implicated in oral candidiasis; and if so, to report potential mechanism of actions. In addition, this review provides an overview of the structure, class, and origin of the diverse classes of polyphenols. To the best of our knowledge, this is the first review that outlines the antifungal activities of various subclasses of flavonoids from pure extracts against C. albicans in vitro, which may lay the foundation for translational use of such novel therapeutics in future in vivo studies as well as in clinical trials.

2. Methods

PubMed, ISI Web of Science, and Scopus were searched systematically for studies published from 2004 until 2015, primarily investigating the antifungal effects of flavonoids on Candida albicans in vitro. Keywords, such as ‘flavonoids/flavones/flavanols/phenolic compounds’ and ‘MIC/in vitro/Candida albicans’ mechanism of action of polyphenols/classification of phenols were used in the databases search. Studies not translated or written in English were restricted using language filters. Abstracts of selected titles were reviewed on the basis of some inclusion and exclusion criteria. For studies to be included: explicit methodology and outcome measurement with specific dose of pure/crude flavonoids on C. albicans had to be reported. Research studies on antifungal activity of compounds without specifying the type of activity or the nature of the respective assay; or publications describing antifungal assay but not stating respective control experiments; as well as studies describing antifungal assay in a contradictory or unclear manner were excluded from the review. Following the search; two authors worked independently to select relevant abstracts for studies analysis. Fig. 1 summarizes the search process and the results obtained following a format suggested by PRISMA guidelines (Liberati et al., 2009).

3. Results and discussion

The initial Pubmed, Web of Science, and Google Scholar search yielded 129, 192, and 90 research papers, respectively. After excluding irrelevant titles and deleting duplicates, 124 abstracts were analyzed based on specific inclusion criteria. From these 124 abstracts, 54 studies were evaluated after eliminating the remaining abstracts based on at least one exclusion criteria. A total of 24 studies that were specific on the antifungal activity of pure extracts of flavonoids against Candida albicans were included in the final analysis of the manuscripts (Fig. 1).

Flavonoids are a class of polyphenols, which are classified according to their biosynthetic origin. The classification of polyphenols presents a challenge, as some classes such as chalcones, flavanones, and flavan-3-ols are both intermediates in biosynthesis process as well as end products accumulating in plant tissues while other classes such as flavones and flavonols are identified as end products in the biosynthesis (Andrae-Marobela, Ghislain, Okatch, & Majinda, 2013). In this paper, we used a classification approach, as outlined by Andrae-Marobela et al., 2013; which classified flavonoids from polyphenols and were subdivided into various subclasses (Fig. 2).

As a major constituents of fruits and vegetables, flavonoids are considered a major class of common plant secondary metabolites and a major group of phytochemicals in the diet, such as apples, onions, wine, and tea (Andrae-Marobela et al., 2013; Maskovic et al., 2011; Moshi, Joseph, Innocent, & Nkunya, 2004; Sitheeque et al., 2009). Their general structure consists of two aromatic rings linked together by a 3-carbon bridge (C2-C3-C4) (Fig. 3a).
Depending on the modifications of the central C-ring, flavonoids can be subdivided into different subclasses, such as flavonols, flavones, flavanols, flavanones, isoflavones, anthocyanidins, and proanthocyanidins (PA) (Uzel et al., 2005). Non-cyclization of the C_3 fragment gives rise to chalcones (Zuzarte et al., 2012), which along with pterocarpanes (an isoflavonoid class) have a different numbering system (Mbaveng et al., 2012b). Flavonoids, including glycosylated derivatives, acylated with phenolic acids, have been identified with more 6000 in plants, and novel compounds are yet to be identified. Flavanols and PAs, also called condensed tannins, are the most abundant and highly complex subclasses among the flavonoids, which are typically found in apples, grapes, berries, and beverages, such as red wine, tea, and cider (Xia et al., 2014).

Considering the relative abundance of flavonoids in the diet composition and its prolonged intake during a life time, health side effects are very minimal, which relate to the bioavailability and metabolism of the compounds (Ziberna et al., 2014). This is reflected by the favorable therapeutic indices of most flavonoids and the low solubility of flavonoids in water. In addition, the low absorption coefficient and the high metabolism of flavonoids contribute to a low risk of acute toxicity (Prasain & Barnes, 2014; Tomás-Navarro et al., 2014; Xia et al., 2014). However, for general safety with respect to therapeutic medical use, flavonoid compounds have to be evaluated in terms of their chemical and pharmacological characteristics (Andrae-Marobela et al., 2013). The antifungal activities of relevant subclasses of flavonoids are summarized in Table 1.

3.1. Subclasses of flavonoids and antifungal activity on Candida albicans

3.1.1. Flavonols

As a major class of flavonoids, flavonols have a 3-hydroxy-flavone backbone structure with different positions of the phenolic —OH group. The specific position of the —OH group contributes to the diversity of this class as well as an increase of the oxidation state, for instance, compared to flavones (Fig. 3b). Members of flavonols include quercetin, myricetin, kaempferol, and galangin. Flavonols are commonly found in apples, bananas, onions, tea, and red wine.

In antifungal assays, C. albicans is the most commonly reported strain primarily due to its high prevalence in oral candidiasis as well as in the disseminated candidiasis, which is considered pathogenic especially in immunocompromised individuals or in patients with an imbalance of the competing bacterial microflora (Lalla et al., 2013; Serpa et al., 2012). The antifungal activity of flavonols; quercetin, myricetin, kaempferol and quercetin derivative 3-O-beta glucoside, against Candida albicans strain have been...
<table>
<thead>
<tr>
<th>Subclass/Compound</th>
<th>Source</th>
<th>Mechanism of Action</th>
<th>Activity</th>
<th>Used Assay/Cell System</th>
<th>References</th>
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<td><strong>Flavonoids</strong></td>
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<tr>
<td>Quercetin</td>
<td>Propolis</td>
<td>Inhibits development of Candida spp.; antifungal activity against subprosthetic stomatitis</td>
<td>MIC = 197 µg/mL to 441 µg/mL</td>
<td>Agar dilution/microdilution</td>
<td>Herrera et al. (2010)</td>
</tr>
<tr>
<td>Myricetin</td>
<td>Propolis</td>
<td>Inhibits development of Candida spp.</td>
<td>MIC = 197 µg/mL to 441 µg/mL</td>
<td>Agar dilution/microdilution</td>
<td>Herrera et al. (2010)</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Propolis</td>
<td>Inhibits development of Candida spp.</td>
<td>MIC = 197 µg/mL to 441 µg/mL</td>
<td>Agar dilution/microdilution</td>
<td>Herrera et al. (2010)</td>
</tr>
<tr>
<td>Pinocembrin</td>
<td>Propolis</td>
<td>Inhibits development of Candida spp.</td>
<td>MIC = 197 µg/mL to 441 µg/mL</td>
<td>Agar dilution/microdilution</td>
<td>Yigit et al. (2009)</td>
</tr>
<tr>
<td>Rutin</td>
<td>Kitaibella vitifolia</td>
<td>Inhibits colony formation</td>
<td>MIC = 7 µg/ml</td>
<td>Paper disc assay</td>
<td>Maskovic et al. (2011)</td>
</tr>
<tr>
<td>Genistein-7-α-L-6-deoxy-talopropanoside (Talosin A)</td>
<td>Kitasatospora kifunensis</td>
<td>Inhibits colony formation</td>
<td>MIC = 15 µg/ml</td>
<td>Paper disc assay</td>
<td>Yoon et al. (2006)</td>
</tr>
<tr>
<td>Genistein-4',7-di-alpha-L-6-deoxy-talopryanoside (talosis B)</td>
<td>Kitasatospora kifunensis</td>
<td>Inhibits colony formation</td>
<td>MIC = 7 µg/ml</td>
<td>Paper disc assay</td>
<td>Taveira et al. (2010)</td>
</tr>
<tr>
<td>Quercetin 3-O-beta-glucoside</td>
<td>Daucus litoralis Smith</td>
<td>Inhibits growth</td>
<td>MIC = 7.8 mg/ml</td>
<td>Microdilution</td>
<td>Yousef beyk et al. (2014)</td>
</tr>
<tr>
<td>Naringenin (R)-roemerine</td>
<td>Propolis</td>
<td>Inhibits growth</td>
<td>MIC = 4 µg/ml</td>
<td>SAR</td>
<td>Uzel et al. (2005)</td>
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<tr>
<td>Galangin</td>
<td>Propolis</td>
<td>Inhibits growth</td>
<td>MIC = 16 µg/mL</td>
<td>SAR</td>
<td>Agnihotri et al. (2008)</td>
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<tr>
<td><strong>Flavones</strong></td>
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<tr>
<td>Baicalin</td>
<td>Scutellaria baicaensis</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
</tr>
<tr>
<td>Robusflavones A</td>
<td>Eriosema robustum</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
</tr>
<tr>
<td>Conzyflavone</td>
<td>Conya canadensis Smith</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
</tr>
<tr>
<td>5,7,3'-tetramethoxyflavone</td>
<td>Kaempferia-parviflora</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
</tr>
<tr>
<td>Smiglabrone A</td>
<td>Smilax glabra</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
</tr>
<tr>
<td>5,7-dihydroxy-flavone</td>
<td>Uvaria scheffleri Dietl</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
</tr>
<tr>
<td>Asterelina A</td>
<td>Asterella angusta</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
</tr>
<tr>
<td>Apigenin</td>
<td>Propolis</td>
<td>Inhibition of efflux pump, induction of apoptosis</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; = 26 µg/ml</td>
<td>In vitro antifungal activity</td>
<td>Serpa et al. (2012)</td>
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<td><strong>Flavanols/Flavan-3-ols</strong></td>
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<tr>
<td>Gallic acid</td>
<td>Paeonia rockii</td>
<td>Inhibits growth, reduction in number of yeast cells and germ tubes</td>
<td>MIC = 30 µg/mL</td>
<td>Micro-broth dilution method, XTT assay and Candida albicans morphological analysis</td>
<td>Picerno et al. (2011)</td>
</tr>
<tr>
<td>Gallotannin</td>
<td>Syzygium cordatum</td>
<td>Inhibits fungal growth</td>
<td>MIC = 0.195 mg/ml</td>
<td>Microdilution bioassay</td>
<td>Mulaudzi et al. (2012)</td>
</tr>
<tr>
<td>1-Galloyl-beta-o-glucopyranosyl-(1→4)-beta-o-galactopyranoside</td>
<td>Baseone-ma acumint-atum</td>
<td>Inhibits fungal growth</td>
<td>MIC = 0.195 mg/ml</td>
<td>Microdilution bioassay</td>
<td>Mulaudzi et al. (2012)</td>
</tr>
<tr>
<td>Catechins</td>
<td>black tea</td>
<td>Inhibits fungal growth</td>
<td>MIC = 0.195 mg/ml</td>
<td>Microdilution bioassay</td>
<td>Mulaudzi et al. (2012)</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Dorstenia mani</td>
<td>Inhibits fungal growth</td>
<td>MIC = 0.195 mg/ml</td>
<td>Microdilution bioassay</td>
<td>Mulaudzi et al. (2012)</td>
</tr>
<tr>
<td>Sedonan A</td>
<td>Dalia farmosa</td>
<td>Inhibition of efflux-mediated pumps, intracellular transcription targets</td>
<td>MIC = 15 µg/ml</td>
<td>Microdilution assay</td>
<td>Mbaveng et al. (2012)</td>
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<tr>
<td><strong>Chalcones</strong></td>
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<tr>
<td>2', 4'- dihydroxy-3'-methoxychalcone</td>
<td>Zuccagnia punctata</td>
<td>Inhibits exoenzymes responsible for invasion mechanisms</td>
<td>MIC = 400 µg/ml</td>
<td>Antifungal activity against planktonic cells</td>
<td>Gabriela et al. (2014)</td>
</tr>
<tr>
<td>2',4'- dihydroxy-chalcone</td>
<td>Zuccagnia punctata</td>
<td>Inhibits biofilm and yeast germ tube formation</td>
<td>MIC = 400 µg/ml</td>
<td>Antifungal activity against planktonic cells</td>
<td>Gabriela et al. (2014)</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>Lavandula multifida</td>
<td>Inhibits fungal growth</td>
<td>MIC = 0.16 µg/ml</td>
<td>Microdilution assay</td>
<td>Zuzarte et al. (2012)</td>
</tr>
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reported by Herrera et al. (2010) and Youssefeyk et al. (2014) (M. Herrera, Alvear, Barrientsos, Montenegro, & Salazar, 2010; Youssefeyk et al., 2014). Isolated from propolis, quercetin inhibited the growth of Candida albicans and exerts an antifungal activity against subprosthesis stomatitis, as reported by Herrera et al., 2010 (Herrera et al., 2010) with MIC values of 197–441 μg/ml using agar microdilution method. Similarly, myrcetin and Kaempferol isolated from the same source propolis, both inhibited Candida species development at MIC values ranging between 197–441 μg/ml (Herrera et al., 2010). Other quercetin derivative, quercetin 3-O-beta glucoside isolated from Daucus littoralis smith inhibited C. albicans growth with an MIC value of 7.8 mg/ml using hepacell carcinoma cells (Youssefeyk et al., 2014). Evaluation of the mechanism of action of genestein-7-alpha-1-deoxy-talipropioiside (Talosin A) isolated from Kitatasospora kifunensis, Yoon, Kim, Kim, Kim, & Suh, (2006) reported inhibition of colony formation C. albicans with an MIC value of 15 μg/ml.

Some studies have employed a structure- activity relationship (SAR) and found flavonols such as (R)-roemerine and Galangin to inhibit the growth of Calbicains at MIC values of 16 μg/ml and 31.2 μg/ml, respectively (Agnihotri et al., 2008; Aguero et al., 2014). Thus, SAR is an important method to determine how variation in chemical structures of molecules may relate to specific biological activities.

3.1.2. Flavones

The general structure of flavones is a keto- group structure at C4 position and a double bond between C2 and C3 (Fig. 3c). Natural occurring flavones include apigenin (4,5,7-trihydroxyflavone), luteolin (3’,4’,5,7-tetrahydroxyflavone), tangeritin (4,6,7,8-pentamethoxyflavone), chrysin (5,7-OH), 6-hydroxyflavone, baicalen (5,6,7-trihydroxyflavone), scutellarein (5,6,7,4’-tetrahydroxyflavone), and wogonin (5,7-OH, 8-OCH3) (Moshi et al., 2004; Serpa et al., 2012; Yenjai, Prasanaph, Daodee, Wongpanich, & Kittakoop, 2004; Yigit et al., 2009). The mechanism of action implicated in this group of compounds is through inhibition of efflux pumps, which ultimately results in induction of cell death or apoptosis (Serpa et al., 2012).

In the subclass of flavones, baicalin isolated from Scutellaria bicaulesis has been reported to inhibit efflux pump and induce apoptosis in C. albicans with an MIC value of 26 μg/ml in an in vitro antifungal activity study (Serpa et al., 2012). It has been noted that Candida cells exposed to baicaelen alone had the presence of blastoconidia of flocculent extracellular material associated with filamentous cells similar to what was observed in scanning electron microscopy (SEM) after the treatment with baicaelen synergistically with fluconazole. In another study by Yenjai et al., 2004 (Yenjai et al., 2004), 5,7,4’-trimethoxyflavone and 5,7,3’,4’-tetramethoxyflavone isolated from Kaempferia parviflora both demonstrated no cytotoxicity against KB, BC and NCI-H187 cell lines and inhibited C. albicans growth at 50% inhibitory concentration (IC50) values of 17.63 μg/ml and 39.71 μg/ml, respectively. Similary, asterin A extracted from asterella angusta inhibited C. albicans growth and colony development at an MIC range of 16–512 μg/ml (Qu, Xie, Guo, Yu, & Lou, 2007). Another flavone compound, apigenin, isolated from propolis demonstrated antifungal activity against C. albicans with an MIC of 197–441 μg/ml in a microdilution assay system (Herrera et al., 2010).

3.1.3. Flavanols/flavan-3-ols

Flavanols or flavan-3-ols are another polyphenol group with a hydroxyl group at position 3 as well as a fully saturated carbon ring structure (Fig. 3d). Compounds that belong to flavan-3-ols are commonly called catechins which are predominantly found in Camellia sinensis include green tea leaves that are made up of catechin, epicatechin (EC), epicatechin gallate (EGC), and epigallocatechin gallate (EGCG) (Fig. 3e, f) (Xia et al., 2014). Green tea has been shown to have antifungal activity against C. albicans, which was found to be pH dependent, with the lowest concentration of 15.6–250 mg/L at pH 7.0 resulting in 90% growth inhibition of C. albicans (Hirasawa & Takada, 2004). Similarly, an in vitro study by Sitheeque et al., 2009 (Sitheeque et al., 2009), catechins found in black tea has inhibited fungal growth with MIC value of 6.25 μg/ml as a result of cell wall damage. Gallic acid isolated from Paonia rockii has been implicated in the reduction of the number of hyphal cells and germ tubes with an MIC of 30 μg/ml against C. albicans in microdilution and morphological analysis studies of C. albicans (Picerno et al., 2011). Other flavanols compounds, such as gallocatechin extracted from Syzygium cordatum, have been shown to have antifungal activity against C. albicans by inhibition of fungal growth at an MIC value of 0.195 mg/ml in a microdilution assay study (Muladuzi, Ndhlala, Kulkarni, & Van Staden, 2012). In addition, it was found that there was synergistic effects of catechins and antimycotics, as Epigallocatechin gallate (EGCG), a known antimicrobial tea polyphenol, was found to inhibit the growth of C. albicans and to enhance the antifungal activity of antimycotics (Chen, Zhai, & Arendrup, 2015; Ning, Ling, & Wu, 2015). In fact, synergism was reported between EGCG and miconazole, fluconazole or amphoterinin B against most both planktonic and biofilm cells of candidias, suggesting that combined treatments of EGCG and conventional antimycotics may result in lower doses administration of antimycotics, and thus minimizing the side effects of such therapeutic agents and the emergence of drug-resistant Candida strains (Ning et al., 2015).

3.1.4. Isoflavones

Some structural variation from flavones are presented in isoflavones, which differs from flavones in the location of the phenyl group at C3 rather than C2 position (Fig. 3g). These compounds are known to act as phytoestrogens because of their close resemblance to estrogen (Forester & Lambert, 2014). Some isoflavones have been known for their antifungal activity, such as glabridin, isolated from Glycyrrhiza glabra, had a broad-spectrum antifungal activity against several Candida species (Liu et al., 2014). Furthermore, glabridin was found to have synergistic effects with fluconazole, as it affected cell membrane permeability, which resulted in cell envelop damage (Liu et al., 2014). In a study by Belovsky et al., 2013; an isoflavone, sedan A isolated from Dalea formosa has inhibited efflux- mediated pumps and affected intracellular transcription targets. Sedon A has inhibited C. albicans and C. glabrata at MIC values of 15 and 7.6 μg/ml, respectively. Another isoflavone known as dorsesmin extracted from dorstenia mannii has been found to have an antifungal activity against C. albicans with an MIC of 64 μg/ml (Mbaveng et al., 2012a).

3.1.5. Chalcones

Chalcones are aromatic ketones composed of two phenyl rings linked together by a 3- carbon structure. They serve as intermediates in the biosynthesis of many other flavonoids (Fig. 3h). Examples of compounds in this group include 2’,4’- dihydroxy-3’-methoxychalcone, 2’,4’- dihydroxychalcone, and carvacrol. Some of the chalcones have been implicated in inhibition of exoenzymes responsible for fungal invasion mechanisms, such as 2’,4’- dihydroxy-3’-methoxychalcone, which in turn exhibited antifungal activity against C. albicans with an MIC of 400 μg/ml (Gabriela et al., 2014). Similarly, in the same study by Gabriela et al., 2014; 2’,4’-dihydroxychalcone, extracted from the same plant source, zuccagnia punctata, inhibited biofilm and germ tube formation in C. albicans (Gabriela et al., 2014). Another chalcone known as carvacrol, has been found to disrupt the cellular cytoplasmic membrane and induce cell apoptosis in several
candida species in an in vitro macrodilution study (Zuzarte et al., 2012).

3.2. Bioavailability and drug interactions of flavonoids

Flavonoids are considered highly bioactive compounds with very low toxicity, which makes them good therapeutic candidates (Prasain & Barnes, 2014). However, low plasma concentrations and cell membrane transporter barriers have been associated with their poor absorption and cellular uptake. It has been noted that tight regulation of flavonoid plasma membrane is accomplished by binding to serum albumin, which acts to lower plasma concentrations of unbound bioactive flavonoids as well as act as a storage system for delayed release into the plasma (Ziberna, Fornasarò, Cvorović, Tramer, & Passamonti, 2014). Albumin-bound flavonoids are stable against oxygen-dependent degradation, which prolongs their biological activity and extends their plasma half-life. Such conditions may protect against high uptake levels of flavonoids and cellular toxicity. In fact, prolonged release of flavonoids from albumin ensures a constant rate of uptake of flavonoids. Ultimately, hydrophobic flavonoids are considered the most bioavailable flavonoids due to the limited uptake along with extensive and fast metabolism (Prasain & Barnes, 2014; Ziberna et al., 2012; Tomás-Narváez et al., 2014).

It has been noted that flavonoids interact with ATP-binding cassette (ABC) efflux transporters, in particular with p-glycoprotein and BCRP (Fukuda & Ashida, 2014). In general, ATP-binding cassette (ABC) transporters (located on the lumens membrane of endothelial cells) or the MRPs are the main efflux transporters responsible for limiting the entry of lipophilic substances into the central nervous system, which have been associated with the decrease of the net cellular absorption of flavonoids and their metabolites (Belosky et al., 2013; Jiang, 2014). Transporters that interact with flavonoids are typically localized at key gateway organs, for example, the stomach, intestine, kidney, liver, and the brain, which can all potentially contribute to the epithelial barrier permeability of flavonoids. Thus, bioavailability of flavonoids is considered to be relatively low, mainly because of the low intestinal permeability and their high metabolism (Ziberna et al., 2014). Isoflavones and catechins flavonones, and quercetin glucosides are considered the best absorbed while condensed tannins, galloylated tea catechins, and anthocyanins are the least absorbed. The interaction of uptake and efflux transporters contribute to the flavonoids passing through the intestinal epithelial barriers. Transporters that are pH-dependent has been suggested in cases with quercetin permeability unlike naringenin absorption which was not pH-dependent. It has been shown that by using specific ABC inhibitors, quercetin efflux is mediated by MRP2 but not by P-gp unlike naringenin which is mediated by both MRP2 and P-gp substrate. MRP2 transporters are located in hepatocytes and are mainly responsible of biliary excretion of flavonoids. Similarly, numerous studies have illustrated the role of MRP2 in flavonoids efflux into the intestinal lumen, such as in the cases of tea flavonoids (EC, ECG, EGC) (Tomás–Navarro, Vallejo, & Tomás–Barberán, 2014).

In terms of investigating multiple flavonoids interaction, few studies have reported additive, opposite, or synergistic effects of combined flavonoids in vivo. For example, antiproliferative activity in vascular smooth muscle cells of four red wine polyphenols; resveratrol, quercetin, ethyl gallate, and (±)-catechin, was stronger than the expected additive effect of each substance used seperately (Sithoeque et al., 2009; Ziberna et al., 2014). One study reported synergistic effect of the flavonoid baicalin (Shi et al., 2010) and flavanone against flavonone-resistant Candida albicans in vitro. Generally speaking, flavonoids increase the bioavailability of co-administered drugs, which in most cases is attributed to inhibitory effects on metabolizing enzymes such as CYP450 isoforms or the efflux transporter P-gp (Belosky et al., 2013; Fukuda & Ashida, 2014; Ziberna et al., 2014). Thus, plasma membrane transporters play a critical role in determining the concentration levels of flavonoids, applied as xenobiotics. A future research direction may involve translating in vitro analysis to in vivo study by understanding the underlying mechanisms of flavonoids pharmacokinetics as well as signaling pathways involved in the binding of flavonoids to plasma membrane and target tissues transporters.

Ultimately, flavonoids show potential antifungal activity against C. albicans, as reported in several studies in the MIC susceptibility assays (Gabriela et al., 2014; Herrera et al., 2010; Picerno et al., 2011; Xu et al., 2013). The specific antifungal mechanism of actions is yet to be elucidated for many of the compounds reported, as several have been reported to generally inhibit colony formation or fungal growth (Mulaudzi et al., 2012; Yoon et al., 2006; Yousef Beyk et al., 2014). However, some studies have alluded to more specific mechanism of actions, such as the case of baicalein, which was found to inhibit efflux pump and induce apoptosis (Serpa et al., 2012). A future research direction may focus on studying several compounds in the same subclass to determine if there may be structural relationship specificity, which may aid in identifying multi-drug targets mechanisms. The significant impact of such may be the translational use of these potentially novel therapeutics in the treatment and/or prevention of oral candidiasis in clinical settings.

**Conflict of interest**

The authors state that they do not have any conflict of interest.

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