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Review

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

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ABSTRACT

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.

Contents

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,

Abbreviations: IC50, 50% inhibitory concentration; MIC, minimum inhibitory concentration; MIC50, 50% minimum inhibitory concentration; SAR, structure activity relationship; MABA, microplate alamar blue assay.

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& Roman, 2016). 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 (Eggimann, 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).

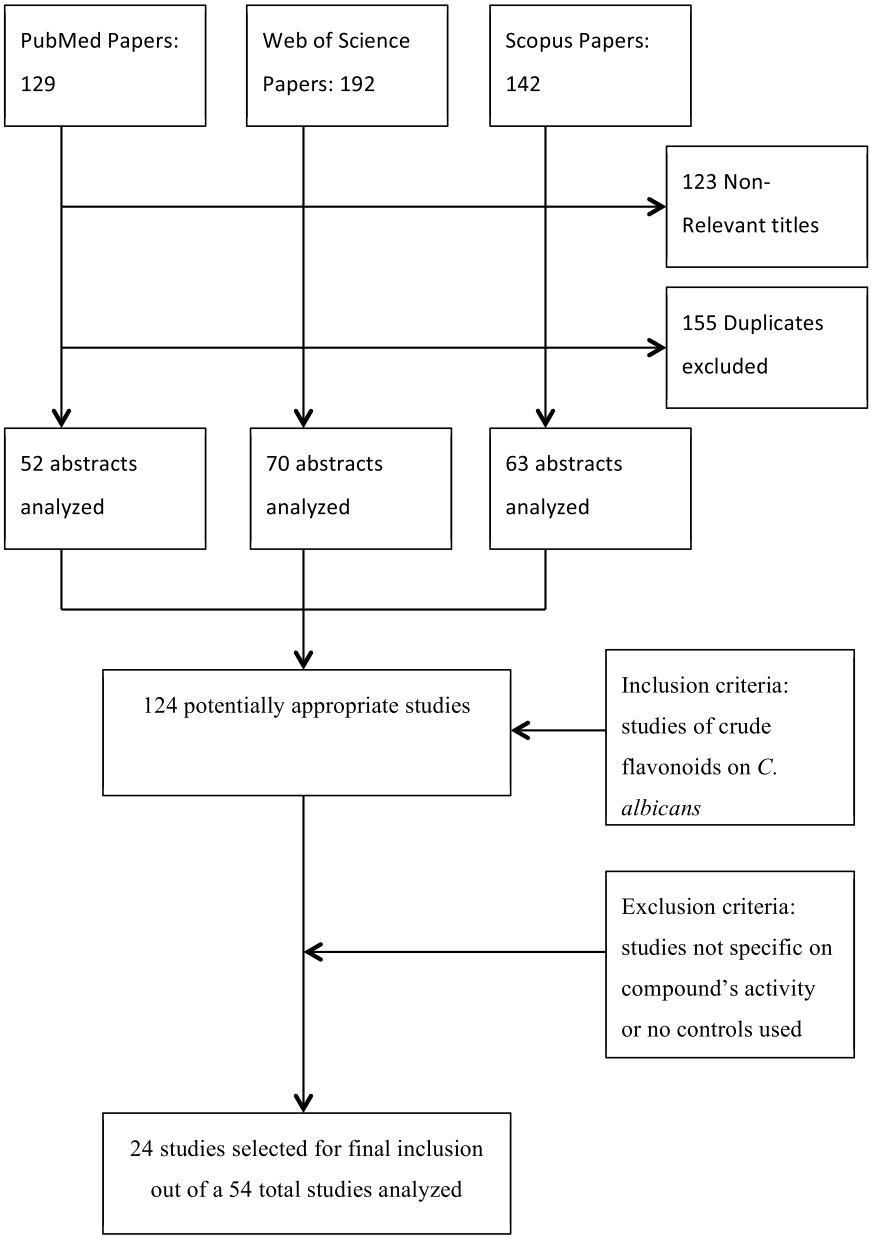

Fig. 1. Search strategy and papers selection flowchart.

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/flavonols/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 PubmMed, 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 criterias. 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 wells 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 (C_6-C_3-C_6) (Fig. 3a).

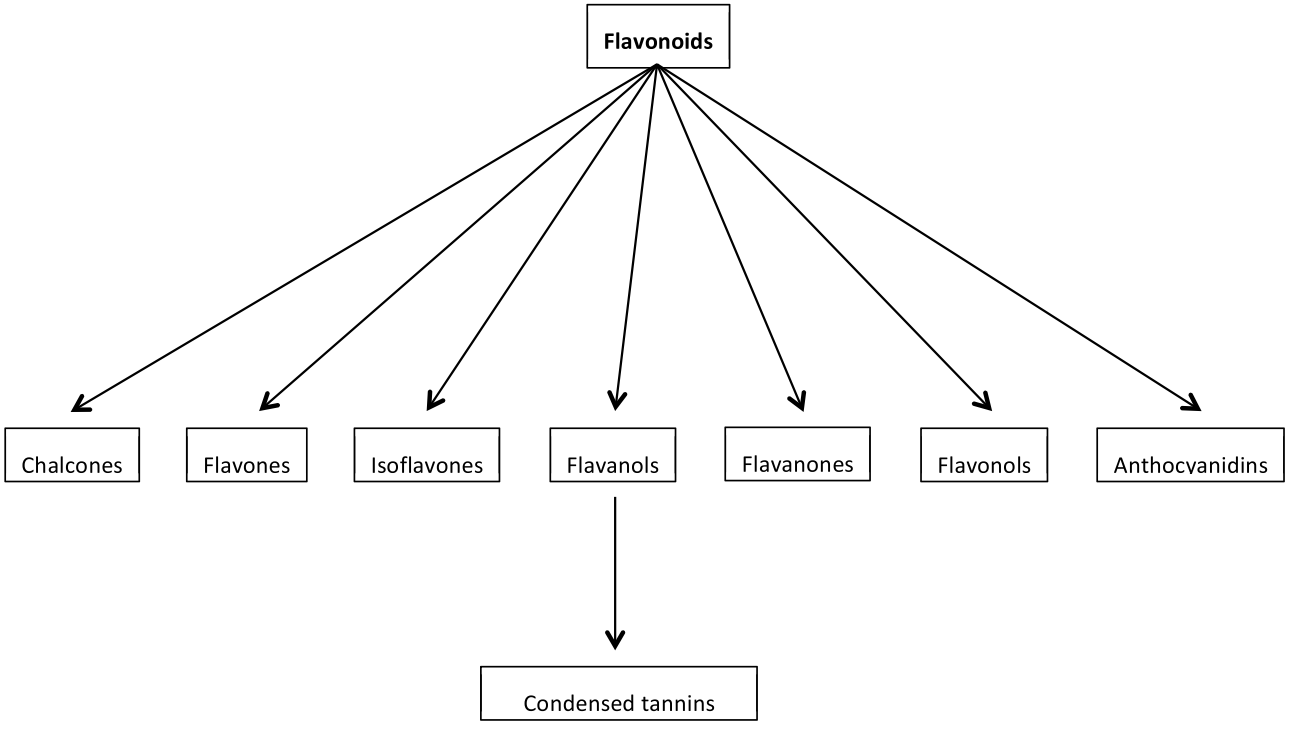

Fig. 2. Classification of flavonoids, a subdivision of polyphenols (from Andrae-Marobela et al., 2013).

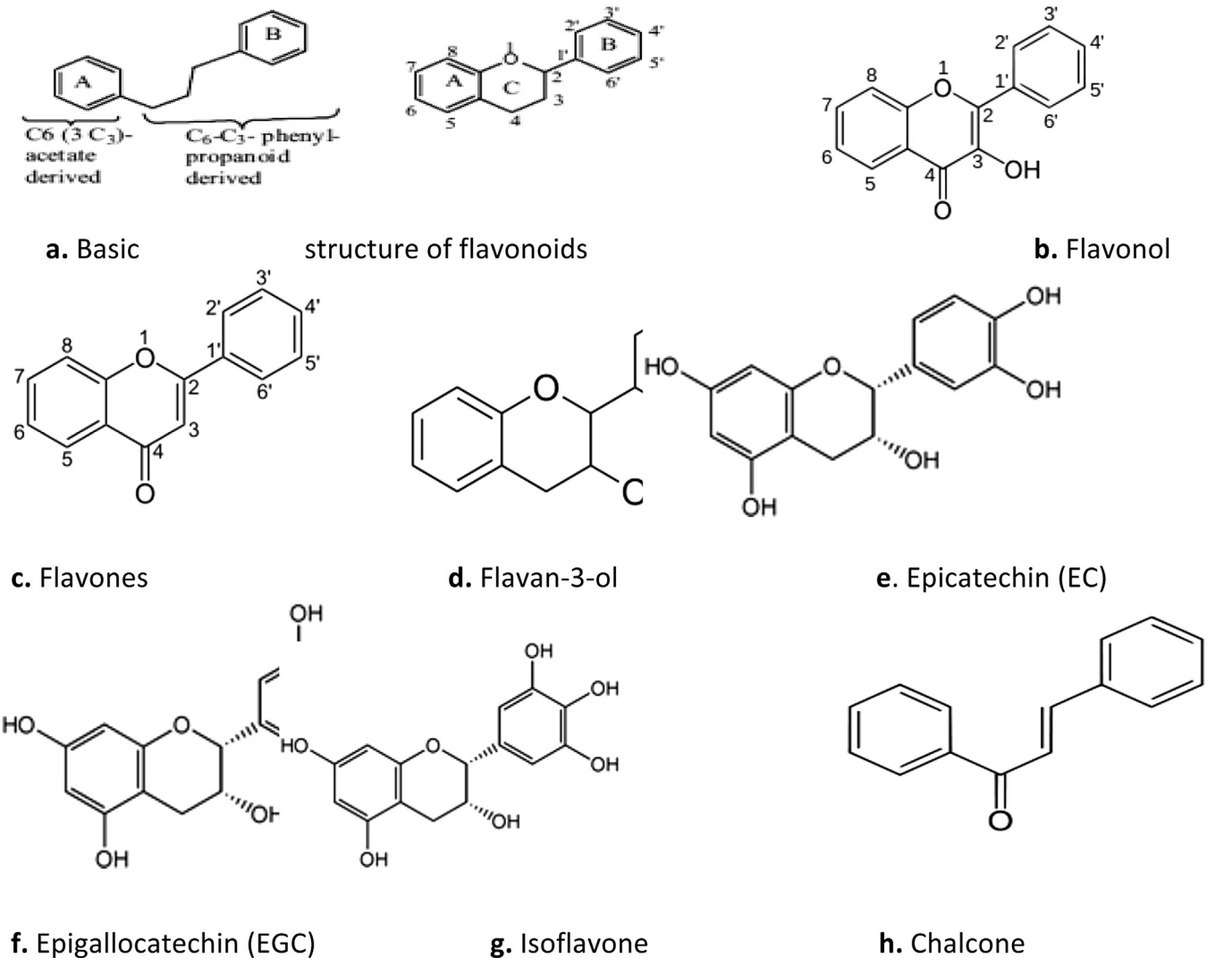

Fig. 3. Structures of representative flavonoids (from Andrae-Marobela et al., 2013).

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 dives rise to chalcones (Zuzarte et al., 2012), which along with pterocarpans (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 mininal, which relate to the bioavailabilty and metabolism of the compounds (Ziberna et al., 2014). This is reflected by the flavorable thaerapeutic 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 toxicty (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 1
Antifungal activities of flavonoids against *C. albicans in vitro.*

Subclass/Compound	Source	Mechanim of Action	Activity	Used Assay/Cell System	References
Flavonols					
Quercetin	*Propolis*	Inhibits development of *Candida spp.;* antifungal activity against subprosthesis stomatitis	MIC = 197 μg/mL to 441 μg/mL	Agar dilution/microdilution	Herrera et al. (2010)
Myricetin	*Propolis*	Inhibits development of *Candida spp.*	MIC = 197 μg/mL to 441 μg/m	Agar dilution/microdilution	Herrera et al. (2010)
Kaempferol	*Propolis*	Inhibits development of *Candida spp.*	MIC = 197 μg/mL to 441 μg/mL	Agar dilution/microdilution	Herrera et al. (2010)
Pinocembrin	*Propolis*	Inhibits development of *Candida spp.*	MIC = 197 μg/mL to 441 μg/m	Agar dilution/microdilution	Yigit et al. (2009)
Rutin	*Kitaibelia vitifolia*		MIC = 15.62 μg/mL	Microdilution/*In vitro* Plates	Maskovic et al. (2011)
Genestein-7-α-L-6- deoxy-taloprinaoside (Talosin A)	*Kitasatos-pora kifunensis*	Inhibits colony formation	MIC = 15 μg/ml	Paper disc assay	Yoon et al. (2006)
Genistein 4',7-di-alpha-L-6-deoxy-talopyranoside (talosin B)	*Kitasatos-pora kifunensis*		MIC = 7 μg/ml	Paper disc assay	Taveira et al. (2010)
Quercetin 3-O-beta-glucoside	*Daucus littoralis Smith*	Inhibits growth	MIC = 7.8 mg/ml	Microdilution	Yousefbeyk et al. (2014)
Naringenin	*Propolis*		MIC = 4 μg/ml	Macrodilution	Uzel et al. (2005)
(R)-roemerine	*Nelumbo nucifera*	Inhibits growth	MIC = 16 μg/mL	SAR	Agnihotri et al. (2008)
Galangin	*Propolis*		MIC = 31.2 μg/mL	SAR	Aguero et al. (2014)
Flavones					
Baicalein	*Scutellaria baicalensis*	Inhibition of efflux pump, induction of apoptosis	MIC_{50} = 26 μg/ml	*In vitro* antifungal activity	Serpa et al. (2012)
Robusflavones A	*Eriosema robustum*		MIC = 0.16 mg/ml	Microdilution, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide reduction assay, Vero cells	Awouafack et al. (2013)
Conyzoflavone	*Conyza canadensis*		MIC = 10 μg/mL	Hole diffusion, macrodilution	Shakirullah et al. (2011)
5,7,3',4'-tetrameth-oxyflavone	*Kaempfer-ia parviflora*		IC50 = 39.71 μg/ml	Antifungal activity, cytotoxicity, KB, BC, and NCI-H187 cell lines	Yenjai et al. (2004)
Smiglabrone A	*Smilax glabra*		MIC = 0.146 mM	SAR, exciton-coupled circular dichroism (ECCD) method	Xu et al. (2013)
5,7-dihydroxy-flavone	*Uvaria scheffleri Diel*	Inhibits development of *Candida spp.*	MIC = 31.25 μg/ml	TLC Bioautographic assay	Moshi et al. (2004)
Asterelin A	*Asterella angusta*		MIC = 16 μg/ml – 512 μg/ml	Bioautographic assay, broth microdilution	Qu et al. (2007)
Apigenin	*Propolis*		MIC = 197 μg/mL to 441 μg/mL	Agar dilution/microdilution	Yigit et al. (2009)
Flavanols/Flavan-3-ols					
Gallic acid	*Paeonia rockii*	Inhibits growth, reduction in number of yeast cells and germ tubes	MIC = 30 μg/ml	Micro-broth dilution method, XTT assay and *Candida albicans* morphological analysis	Picerno et al. (2011)
Gallotannin	*Syzygium cordatum*	Inhibits fungal growth	MIC = 0.195 mg/ml	Microdilution bioassay	Mulaudzi et al. (2012)
1-Galloyl-beta-D-glucopyranosyl-(1→4)-beta-D-galactopyranoside	*Baseone-ma acumin-atum*		MIC = 12.5 μg/mL	*In vitro* antimicrobial activity	De Leo et al. (2004)
Catechins	black tea	Cell wall damage	MIC = 6.25 μg/ml	*In vitro* agar diffusion growth inhibition assay	Sitheeque et al. (2009)
Isoflavones					
Dorsmanin	*Dorstenia manni*		MIC = 64 μg/ml	Broth microdilution, MABA	Mbaveng et al. (2012a,b)
Sedonan A	*Dalea formosa*	Inhibition of efflux-mediated pumps, intracellular transcription targets	MIC = 15 μg/ml	Microdilution assay	Belofsky et al. (2013)
Chalcones					
2′, 4′- dihydroxy-3′-methoxychalcone	*Zuccagnia punctata*	Inhibits exoenzymes responsible for invasion mechanisms	MIC = 400 μg/ml	Antifungal activity against planktonic cells	Gabriela et al. (2014)
2′,4′- dihydrocychalcone	*Zuccagnia punctata*	Inhibits biofilm and yeast germ tube formation	MIC = 400 μg/ml	Antifungal activity against planktonic cells	Gabriela et al. (2014)
Carvacrol	*Lavandula multifida*	Cytoplasmic membrane disruption, cell death induction, inhibits filamentat-ion	MIC = 0.16 μL/ml	Broth macrodilution flow cytometry	Zuzarte et al. (2012)

reported by Herrera et al. (2010) and Yousefbeyk et al. (2014) (M. Herrera, Alvear, Barrientos, Montenegro, & Salazar, 2010; Yousefbeyk 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 *Daucaus littoralis smith* inhibited *C. albicans* growth with an MIC value of 7.8 mg/ml using hepacellular carcinoma cells (Yousefbeyk et al., 2014). Evaluation of the mechanism of action of genestein-7-α-L-6-deoxy-taloprinaoside (Talosin A) isolated from *Kitasatospora 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 stucture- activity relationship (SAR) and found flavonols such as (R)-roemerine and Galangin to inhibit the growth of *C.albicans* 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 occuring flavones include apigenin (4′,5,7-trihydroxyflavone), luteolin (3′,4′,5,7-tetrahydroxyflavone), tangeritin (4′,5,6,7,8-pentamethoxyflavone), chrysin (5,7-OH), 6-hydroxyflavone, baicalein (5,6,7-trihydroxyflavone), scutellarein (5,6,7,4′-tetrahydroxyflavone), and wogonin (5,7-OH, 8-OCH_3) (Moshi et al., 2004; Serpa et al., 2012; Yenjai, Prasanphen, Daodee, Wongpanich, & Kittakoop, 2004; Yigit et al., 2009). The mechansim 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, baicalein isolated from *Scutellaria biacalensis* has been reported to inhibit efflux pump and induce appoptosis 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 baicalein 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 baicalein synergistically with fluconazole. In another study by Yenjai et al., 2004 (Yenjai et al., 2004), 5,7,4′-trimethoxy-flavone and 5,7,3′,4′-tetrameth-oxyflavone 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, asterlin A extracted from *asterella angusta* inhibited C. albicans growth and colony development at an MIC rannge of 16–512 μg/ml (Qu, Xie, Guo, Yu, & Lou, 2007). Another flavone compound, apigenein, 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 (ECG), 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 *Paeonia 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 gallotannin 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 (Mulaudzi, 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 amphotericin B against most both planktonic and biofilm cells of *Candida*, 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, whch 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, glabdrin 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 Belofsky et al., 2013; an isoflavone, sedonan A isolated from *Dalea formosa* has inhibited efflux- mediated pumps and affected intracellular transcription targets. Sedonan A has inhibited *C. albicans* and *C. glabrata* at MIC values of 15 and 7.6 μg/ml, espectively. Another isoflavone known as dorsmanin 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, Fornasaro, Čvorović, 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 flavoinoids. 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., 2014; Tomás-Navarro 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 lumen membrane of endothelial cells) or the MRP's 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 (Belofsky 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, bioavailibility 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 flavanones, 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 epitheilial 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 flvonoids (EC, ECG, EGCG) (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, antiprofilerative 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 (Sitheeque et al., 2009; Ziberna et al., 2014). One study reported synergistic effect of the flavonoid baicalein (Shi et al., 2010) and flucanozole against fluconazole-resistant *Candida albicans in vitro*. Generally speaking, flavonoids increase the bioavailiability of co-adminstered drugs, which in most cases is attributed to inhibitory effects on metabolizying enzymes such as CYP450 isoforms or the efflux transporter P-gp (Belofsky 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 pharmokinetics 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 suscepitibility 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; Yousefbeyk 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 signficant 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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References

Agnihotri, V. J. K., ElSohly, H. N., Khan, S. I., Jacob, M. R., Joshi, V. C., Smillie, T., & Walker, L. A. (2008). Constituents of Nelumbo nucifera leaves and their antimalarial and antifungal activity. *Phytochemistry Letters*, *1*(2), 89–93. http://dx.doi.org/10.1016/j.phytol.2008.03.003.

Aguero, M. B., Svetaz, L., Baroni, V., Lima, B., Luna, L., Zacchino, S., ... Tapia, A. (2014). Urban propolis from San Juan province (Argentina): Ethnopharmacological uses and antifungal activity against Candida and dermatophytes. *Industrial Crops and Products*, *57*, 166–173. http://dx.doi.org/10.1016/j.indcrop.2014.03.009.

Andrae-Marobela, K., Ghislain, F. W., Okatch, H., & Majinda, R. R. (2013). Polyphenols: A diverse class of multi-target anti-HIV-1 agents. *Current Drug Metabolism*, *14*(4), 392–413.

Awouafack, M. D., McGaw, L. J., Gottfried, S., Mbouangouere, R., Tane, P., Spiteller, M., et al. (2013). Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from *Eriosema robustum* (*Fabaceae*). *BMC Complementary and Alternative Medicine*, *13*, 9.

Belofsky, G., Kolaczkowski, M., Adams, E., Schreiber, J., Eisenberg, V., Coleman, C. M., ... Ferreira, D. (2013). Fungal ABC transporter-associated activity of isoflavonoids from the root extract of Dalea formosa. *Journal of Natural Products*, *76*(5), 915–925. http://dx.doi.org/10.1021/np4000763.

Borg-von Zepelin, M., Meyer, I., Thomssen, R., Wurzner, R., Sanglard, D., Telenti, A., & Monod, M. (1999). HIV-Protease inhibitors reduce cell adherence of Candida albicans strains by inhibition of yeast secreted aspartic proteases. *Journal of Investigative Dermatology*, *113*(5), 747–751. http://dx.doi.org/10.1046/j.1523-1747.1999.00747.x.

Chen, M., Zhai, L., & Arendrup, M. C. (2015). In vitro activity of 23 tea extractions and epigallocatechin gallate against Candida species. *Medical Mycology*, *53*(2), 194–198. http://dx.doi.org/10.1093/mmy/myu073.

Darwazeh, A. M., Hammad, M. M., & Al-Jamaei, A. A. (2010). The relationship between oral hygiene and oral colonization with Candida species in healthy adult subjects. *International Journal of Dental Hygiene*, *8*(2), 128–133. http://dx.doi.org/10.1111/j.1601-5037.2009.00407.x.

Das, I., Nightingale, P., Patel, M., & Jumaa, P. (2011). Epidemiology, clinical characteristics, and outcome of candidemia: Experience in a tertiary referral center in the UK. *International Journal of Infectious Diseases*, *15*(11), e759–e763. http://dx.doi.org/10.1016/j.ijid.2011.06.006.

De Leo, M., Braca, A., De Tommasi, N., Norscia, I., Morelli, I., Battinelli, L., et al. (2004). Phenolic compounds from *Baseonema acuminatum* leaves: isolation and antimicrobial activity. *Planta Medica*, *70*, 841–846.

Eggimann, P., Que, Y. A., Revelly, J. P., & Pagani, J. L. (2015). Preventing invasive candida infections. Where could we do better? *Journal of Hospital Infection*, *89*(4), 302–308. http://dx.doi.org/10.1016/j.jhin.2014.11.006.

Forester, S. C., & Lambert, J. D. (2014). Chapter 98—Cancer preventive effects of green tea polyphenols. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (pp. 1309–1322).San Diego: Academic Press.

Fukuda, I., & Ashida, H. (2014). Chapter 86—Modulation of drug-Metabolizing enzymes and transporters by polyphenols as an anticarcinogenic effect. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (pp. 1127–1135).San Diego: Academic Press.

Gabriela, N., Rosa, A. M., Catiana, Z. I., Soledad, C., Mabel, O. R., Esteban, S. J., & Ines, I. M. (2014). The effect of zuccagnia punctata, an argentine medicinal plant, on virulence factors from Candida species. *Natural Product Communications*, *9*(7), 933–936.

Gillies, M., Ranakusuma, A., Hoffmann, T., Thorning, S., McGuire, T., Glasziou, P., & Del Mar, C. (2015). Common harms from amoxicillin: A systematic review and meta-analysis of randomized placebo-controlled trials for any indication. *CMAJ*, *187*(1), E21–E31. http://dx.doi.org/10.1503/cmaj.140848.

Herrera, C. L., Alvear, M., Barrientos, L., Montenegro, G., & Salazar, L. A. (2010). The antifungal effect of six commercial extracts of Chilean propolis on Candida spp. *Ciencia E Investigacion Agraria*, *37*(1), 75–84.

Hirasawa, M., & Takada, K. (2004). Multiple effects of green tea catechin on the antifungal activity of antimycotics against Candida albicans. *Journal of Antimicrobial Chemotherapy*, *53*(2), 225–229. http://dx.doi.org/10.1093/jac/dkh046.

Jiang, F. (2014). Chapter 73—Pharmacology of polyphenols in cardiac remodeling: Potential biological mechanisms. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (pp. 947–962).San Diego: Academic Press.

Kamba, A. S., & Hassan, L. G. (2010). Phytochemical screening and antimicrobial activities of Euphorbia balsamifera leaves, stems and root against some pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*, *4*(9), 645–652.

Lalla, R. V., Patton, L. L., & Dongari-Bagtzoglou, A. (2013). Oral candidiasis: Pathogenesis, clinical presentation, diagnosis and treatment strategies. *Journal of the California Dental Association*, *41*(4), 263–268.

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gotzsche, P. C., Ioannidis, J. P., . . . Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. *Bmj*, *339*, b2700. http://dx.doi.org/10.1136/bmj.b2700.

Liu, W., Li, L. P., Zhang, J. D., Li, Q., Shen, H., Chen, S. M., & Jiang (2014). Synergistic antifungal effect of glabridin and fluconazole. *PLoS One*, *9*(7), e103442. http://dx.doi.org/10.1371/journal.pone.0103442.

Maskovic, P., Solujic, S., Mihailovic, V., Mladenovic, M., Cvijovic, M., Mladenovic, J., . . . Kurcubic, V. (2011). Phenolic compounds and biological activity of Kitaibelia vitifolia. *Journal of Medicinal Food*, *14*(12), 1617–1623. http://dx.doi.org/10.1089/jmf.2011.0013.

Mbaveng, A. T., Kuete, V., Ngameni, B., Beng, V. P., Ngadjui, B. T., Meyer, J. J., & Lall, N. (2012a). Antimicrobial activities of the methanol extract and compounds from the twigs of Dorstenia mannii (Moraceae). *BMC Complementary and Alternative Medicine*, *12*, 83. http://dx.doi.org/10.1186/1472-6882-12-83.

Mbaveng, A. T., Kuete, V., Ngameni, B., Beng, V. P., Ngadjui, B. T., Meyer, J. J. M., & Lall, N. (2012b). Antimicrobial activities of the methanol extract and compounds from the twigs of Dorstenia mannii (Moraceae). *BMC Complementary and Alternative Medicine*, *12*, . http://dx.doi.org/10.1186/1472-6882-12-83.

Moshi, M. J., Joseph, C. C., Innocent, E., & Nkunya, M. H. H. (2004). In vitro antibacterial and antifungal activities of extracts and compounds from Uvaria scheffleri. *Pharmaceutical Biology*, *42*(4–5), 269–273. http://dx.doi.org/10.1080/13880200490511035.

Mulaudzi, R. B., Ndhlala, A. R., Kulkarni, M. G., & Van Staden, J. (2012). Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. *Journal of Ethnopharmacology*, *143*(1), 185–193. http://dx.doi.org/10.1016/j.jep.2012.06.022.

Ning, Y., Ling, J., & Wu, C. D. (2015). Synergistic effects of tea catechin epigallocatechin gallate and antimycotics against oral Candida species. *Archives of Oral Biology*, *60*(10), 1565–1570. http://dx.doi.org/10.1016/j.archoralbio.2015.07.001.

Picerno, P., Mencherini, T., Sansone, F., Del Gaudio, P., Granata, I., Porta, A., & Aquino, R. P. (2011). Screening of a polar extract of Paeonia rockii: Composition and antioxidant and antifungal activities. *Journal of Ethnopharmacology*, *138*(3), 705–712. http://dx.doi.org/10.1016/j.jep.2011.09.056.

Prasain, J. K., & Barnes, S. (2014). Chapter 41—Uptake and metabolism of dietary proanthocyanidins. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (pp. 553–560).San Diego: Academic Press.

Prieto, D., Correia, I., Pla, J., & Roman, E. (2016). Adaptation of Candida albicans to commensalism in the gut. *Future Microbiology*. http://dx.doi.org/10.2217/fmb.16.1.

Qu, J. B., Xie, C. F., Guo, H. F., Yu, W. T., & Lou, H. X. (2007). Antifungal dibenzofuran bis (bibenzyl)s from the liverwort Asterella angusta. *Phytochemistry*, *68*(13), 1767–1774. http://dx.doi.org/10.1016/j.phytochem.2007.04.036.

Sanguinetti, M., Posteraro, B., & Lass-Florl, C. (2015). Antifungal drug resistance among Candida species: Mechanisms and clinical impact. *Mycoses*, *5*(Suppl. 2), 2–13. http://dx.doi.org/10.1111/myc.12330.

Serpa, R., Franca, E. J., Furlaneto-Maia, L., Andrade, C. G., Diniz, A., & Furlaneto, M. C. (2012). In vitro antifungal activity of the flavonoid baicalein against Candida species. *Journal of Medical Microbiology*, *61*(Pt. 12), 1704–1708. http://dx.doi.org/10.1099/jmm.0.047852-0.

Shakirullah, M., Ahmad, H., Shah, M. R., Ahmad, I., Ishaq, M., Khan, N., et al. (2011). Antimicrobial activities of Conyzolide and Conyzoflavone from *Conyza canadensis*. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *26*, 468–471.

Shi, W., Chen, Z., Chen, X., Cao, L., Liu, P., & Sun, S. (2010). The combination of minocycline and fluconazole causes synergistic growth inhibition against Candida albicans: An in vitro interaction of antifungal and antibacterial agents. *FEMS Yeast Research*, *10*(7), 885–893. http://dx.doi.org/10.1111/j.1567-1364.2010.00664.x.

Sitheeque, M. A., Panagoda, G. J., Yau, J., Amarakoon, A. M., Udagama, U. R., & Samaranayake, L. P. (2009). Antifungal activity of black tea polyphenols (catechins and theaflavins) against Candida species. *Chemotherapy*, *55*(3), 189–196. http://dx.doi.org/10.1159/000216836.

Tang, H. J., Liu, W. L., Lin, H. L., & Lai, C. C. (2014). Epidemiology and prognostic factors of candidemia in cancer patients. *PLoS One*, *9*(6), e99103. http://dx.doi.org/10.1371/journal.pone.0099103.

Taveira, M., Silva, L. R., Vale-Silva, L. A., Pinto, E., Valentao, P., Ferreres, F., . . . Andrade, P. B. (2010). Lycopersicon esculentum seeds: An industrial byproduct as an antimicrobial agent. *Journal of Agricultural and Food Chemistry*, *58*(17), 9529–9536. http://dx.doi.org/10.1021/jf102215g.

Tomás-Navarro, M., Vallejo, F., & Tomás-Barberán, F. A. (2014). Chapter 40—Bioavailability and metabolism of citrus fruit beverage flavanones in humans. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (pp. 537–551).San Diego: Academic Press.

Toure, A., Bahi, C., Ouattara, K., Djama, J. A., & Coulibaly, A. (2011). Phytochemical screening and in vitro antifungal activities of extracts of leaves of Morinda morindoides (Morinda, Rubiaceae). *Journal of Medicinal Plants Research*, *5*(31), 6780–6786. http://dx.doi.org/10.5897/jmpr11.1116.

Uzel, A., Sorkun, K., Oncag, O., Cogulu, D., Gencay, O., & Salih, B. (2005). Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. *Microbiological Research*, *160*(2), 189–195.

Xia, E., He, X., Li, H., Wu, S., Li, S., & Deng, G. (2014). Chapter 5— biological activities of polyphenols from grapes. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (pp. 47–58).San Diego: Academic Press.

Xu, S., Shang, M. Y., Liu, G. X., Xu, F., Wang, X., Shou, C. C., & Cai, S. Q. (2013). Chemical constituents from the rhizomes of Smilax glabra and their antimicrobial activity. *Molecules*, *18*(5), 5265–5287. http://dx.doi.org/10.3390/molecules18055265.

Yenjai, C., Prasanphen, K., Daodee, S., Wongpanich, V., & Kittakoop, P. (2004). Bioactive flavonoids from Kaempferia parviflora. *Fitoterapia*, *75*(1), 89–92. http://dx.doi.org/10.1016/j.fitote.2003.08.017.

Yigit, D., Yigit, N., & Mavi, A. (2009). Antioxidant and antimicrobial activities of bitter and sweet apricot (Prunus armeniaca L.) kernels. *Brazilian Journal of Medical and Biological Research*, *42*(4), 346–352.

Yoon, T. M., Kim, J. W., Kim, J. G., Kim, W. G., & Suh, J. W. (2006). Talosins A and B: New isoflavonol glycosides with potent antifungal activity from Kitasatospora kifunensis MJM341 I. Taxonomy, fermentation, isolation, and biological activities. *Journal of Antibiotics (Tokyo)*, *59*(10), 633–639. http://dx.doi.org/10.1038/ja.2006.84.

Yousefbeyk, F., Gohari, A. R., Hashemighahderijani, Z., Ostad, S. N., Salehi Sourmaghi, M. H., Amini, M., . . . Amin, G. (2014). Bioactive terpenoids and flavonoids from Daucus littoralis Smith subsp. hyrcanicus Rech.f, an endemic species of Iran. *Daru*, *22*(1), 12. http://dx.doi.org/10.1186/2008-2231-22-12.

Ziberna, L., Fornasaro, S., Čvorović, J., Tramer, F., & Passamonti, S. (2014). Chapter 37—Bioavailability of flavonoids: The role of cell membrane transporters. In R. R. Watson, V. R. Preedy, & S. Zibadi (Eds.), *Polyphenols in human health and disease* (pp. 489–511).San Diego: Academic Press.

Zuzarte, M., Vale-Silva, L., Goncalves, M. J., Cavaleiro, C., Vaz, S., Canhoto, J., . . . Salgueiro, L. (2012). Antifungal activity of phenolic-rich Lavandula multifida L. essential oil. *European Journal of Clinical Microbiology & Infectious Diseases*, *31*(7), 1359–1366. http://dx.doi.org/10.1007/s10096-011-1450-4.