ANTI-*CANDIDA* ACTIVITY OF FLAVONOIDS - AN OVERVIEW

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Abstract

Flavonoids are a group of plant polyphenols which received an increased attention during the recent past due to their important antimicrobial activities. Those compounds could be a reliable source of new antifungals, used to efficiently control infections caused by pathogenic fungi such as *Candida spp. Candida* species represents a leading cause of mortality all around the world, posing a serious threat to medical systems. Therefore, finding new compounds with antifungal activity for treatment of *Candida* infections is a real challenge of modern medicine. This review focuses on the antifungal activity of natural, semi-synthetic and synthetic flavonoids against the most prevalent pathogenic *Candida* species. In addition, the review outlines the mechanisms of action and the possible use of flavonoids as anti-virulence agents to withstand *Candida* pathogenicity and antifungal resistance.
Keywords: flavonoids, *Candida*, fungistatic, fungicidal, anti-virulence agents, anti-biofilm activity, antifungal resistance

Introduction

Candida species represent an important cause of morbidity and mortality globally, posing a serious threat to medical systems, especially among immunocompromised patients (de Oliveira Santos et al. 2018). Recent data are showing that approximately 250,000 patients are affected by candidiasis each year (Arendrup and Patterson 2017). *Candida albicans* is considered the main cause of infections, but other species (non-albicans) such as *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis* emerge as opportunistic pathogens with increased mortality rates in bloodstream infections (Al-Musawi et al. 2021).

The treatment of candidiasis is becoming more and more difficult since many pathogenic *Candida* strains acquire resistance following exposure to different conventional antifungal drugs such as polyenes, azoles, allylamines, echinocandins or triterpenoids. In addition, some *Candida* species like *C. krusei* are naturally resistant or less susceptible to different antifungals (Jamiu et al. 2021). Therefore, finding new antifungal agents to be used in effective *Candida* infections control is a real challenge of modern medicine (de Oliveira Santos et al. 2018).

Flavonoids are a class of plant low-molecular-weight polyphenols which occur as aglycones, glycosides and methylated derivatives (Narayana et al. 2001). Depending on their structure, flavonoids are classified in different categories, such as flavones, flavonols, flavanones and others (Kumar and Pandey 2013). Flavonoids were used for centuries in traditional medicine due to their important biological activities, such as antioxidant, antitumoral, anti-inflammatory or cardioprotective (Sarbu et al. 2019). Also, they received increasing interest over the last decades because of their important antiviral, antibacterial and antifungal activity.

The antifungal activity of flavonoids has been extensively documented in literature (Jin 2019). However, few papers refer to the anti-*Candida* activity of flavonoids, such as the studies of (Seleem et al. 2017, Al Aboody and Mickymaray 2020, Nguyen et al. 2021). More information

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is available about the anti-*Candida* potential of natural flavonoids and less is documented about the semi-synthetic or synthetic flavonoids. Therefore, the main goal of this review is to evaluate the antifungal activity of natural, semi-synthetic and synthetic flavonoids against the most prevalent *Candida* species. In addition, this review outlines the mechanisms of action and flavonoids effect against different factors involved in *Candida* pathogenicity and resistance to conventional antifungals.

The identification of flavonoids with high anti-*Candida* activity at low concentrations could offer efficient alternatives to the clinical use of conventional antifungal drugs for which the resistance is already displayed. Moreover, combinations of flavonoids with available drugs could be an attractive strategy to increase the therapeutic efficiency and to reduce the cytotoxicity and side effects. Therefore, the information presented here may be relevant for the development of new effective drugs used to treat candidiasis and to overcome the alarming increase of antimicrobial resistance.

1. Flavonoids as fungistatic agents

One of the most used microbiological parameters employed to assess the anti-*Candida* activity of flavonoids is the minimum inhibitory concentration (MIC), estimated using serial dilution methods. MIC is considered the lowest concentration of a compound with antifungal properties capable of inhibiting fungal growth. Usually, MIC values are related to the resistance or sensitivity levels of fungal strains to different concentrations of tested antifungals. The proper assessment of the MIC has a particular practical importance in choosing the most effective antifungal for the therapy of candidiasis (Kowalska-Krochmal and Dudek-Wicher 2021).

The MIC values of reported flavonoids against different *Candida* species are summarized in Table 1. The lowest minimum inhibitory concentration presented in this review for natural flavonoids (0.31 µg/mL) was recorded for epigallocatechin gallate against *C. glabrata* ATCC 2001 strain by Chen et al. The flavonoid also exhibited an important activity against *C. albicans* ATCC 2091, with a reported MIC value of 5 µg/mL (Chen et al. 2015).

A remarkable fungistatic activity was registered also for quercetin, with MIC values of 0.5 µg/mL against *C. parapsilosis sensu stricto* and 2 µg/mL against *C. krusei* ATCC 6258 (Rocha et al. 2019). Tested against *C. glabrata* strains (including *C. glabrata* 510), quercetin showed an important growth inhibition potential with MIC values of 15.6 µg/mL (Salazar-Aranda et al. 2015) and 64 µg/mL (Sadeghi-Ghadi et al. 2020). The inhibition of *C. albicans* 475 growth occurred when fungal cells were exposed to quercetin at concentrations of 75 µg/mL (Ivanov et al. 2020).

Among natural flavonoids, baicalein showed one of the lowest fungistatic activity against *Candida* pathogens. Thus, MIC values as low as 1.28 mg/mL were reported against *C. albicans* strains (Liu et al. 2017). Baicalein also inhibited the growth of *C. parapsilosis* and *C. krusei* with corresponding MICs of 350 µg/mL and 400 µg/mL (Kvasnickova et al. 2015). However, recent data are showing that baicalein has important fungistatic potential against *C. albicans* ATCC 10231 with a reported MIC of 16 µg/mL (Janeczko et al. 2022).

Isoquercitrin isolated from *Aster yomena* showed remarkable activity against *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 strains, with a MIC of 2.5 µg/mL (Yun et al. 2015). In a more recent study conducted in 2020, Ivanov et al. reported isoquercitrin as a less effective fungistatic agent against *C. albicans* 475, with a MIC value of 37.5 µg/mL (Ivanov et al. 2020).

Similar MIC values (3.9 µg/mL) were also recorded by Salazar et al. for luteolin against *C. glabrata* 493 (Salazar-Aranda et al. 2015). The flavonoid was less effective against *C. albicans* 475, with a MIC of 37.5 µg/mL (Ivanov et al. 2020).

Glabridin, a flavonoid extracted from *Glycyrrhiza glabra* roots, exhibited also important anti-*Candida* activity at concentrations as low as 4 and 8 µg/mL (Moazeni et al. 2017).

In the study of Gao et al., the antifungal activity of quercetin against *C. albicans* ATCC 10231 was characterized by a high MIC value (512 µg/mL), although other authors reported this flavonoid as an important fungistatic agent against non-*Candida albicans* species, as we showed above (Gao et al. 2016).
Antimicrobial research is focused nowadays more on flavonoid derivatives, compounds with higher antifungal activity compared to flavonoids isolated from natural sources. Thus, two chalcone derivatives were found to have significant activity against *C. albicans* 7535, inhibiting fungal growth at concentrations as low as 1 µg/mL (Wei et al. 2016).
An important inhibitory activity against *C. albicans* strains was also exhibited by two new dehydroacetic acid chalcone-1,2,3-triazole hybrids, 5n and 5o, with MIC values of 0.0034 µM/mL and 0.0062 µM/mL, respectively (Lal et al. 2018).
Some derivatives of mono- and bis-chalcones showed important antifungal activity against resistant *C. albicans* isolates, with MICs of 0.5 µg/mL and 2 µg/mL (Ahmad et al. 2017).
Potent activities against *Candida* strains were also reported for imidazole–chalcone derivative with MICs as low as 0.78 µg/mL (Osmaniye et al. 2018).
Synthetic flavonoids derived from chalcone/amine such as compounds 36, 37, 38 and 42 significantly inhibited *C. albicans* IFO 0583 growth at concentrations of 2 µg/mL, 3 µg/mL and 4 µg/mL (El-Messery et al. 2018).
4′-fluoro-3-(1,2,4-triazol-1-yl) flavanones showed an improved antifungal activity compared to conventional antimycotics against *Candida* at concentrations of 3.9 µg/mL (Emami et al. 2013).
Siddiqui et al. reported important fungistatic activity of chromonyl chalcones against *C. albicans*, with MIC of 12.5 µg/mL (Siddiqui et al. 2012).
A new fluorine substituted chalcone analogs (3) exerted a fungistatic effect on *C. albicans*, *C. glabrata* and *C. parapsilosis* cells exposed to concentrations of 15.62 µg/mL, 31.25 µg/mL and 62.5 µg/mL (Burmaoglu et al. 2017).
Other synthetic flavonoids such as those derived from baicalin ester (2a, 2d, 2e, 2f and 2g) have activity against *C. albicans* ATCC 10231, with MIC values between 0.4 µM and 6.4 µM (Xin et al. 2019).
Tamfu et al. showed that triacontyl *p*-coumarate, a new isoflavonol, has antifungal activity against *C. krusei*, *C. glabrata*, *C. albicans* and *C. parapsilosis* strains with MIC values between 125 µg/mL and 500 µg/mL (Tamfu et al. 2020).
An important anti-*Candida* activity has also been reported by our research group for a novel synthetic flavonoid with bromine as substituent at the benzopyran core named BrCl-flav. Thus, we showed that flavonoid BrCl inhibited the growth of clinical isolates *C. albicans* Prx, *C. parapsilosis* Prx, *C. glabrata* Cam, *C. krusei* Prx strains at a concentration of 15.62 µg/mL (Babii et al. 2021).

Table 1. The MIC values of reported flavonoids against different *Candida* species

Flavonoid	*Candida* sp.	MIC value	Reference
Natural flavonoids			
Isoquercitrin	*C. albicans* ATCC 90028	2.5 µg/mL	(Yun et al. 2015)
Isoquercitrin	*C. parapsilosis* ATCC 22019	2.5 µg/mL	
Isoquercitrin	*C. albicans* 475	37.5 µg/mL	(Ivanov et al. 2020)
Baicalein	*C. parapsilosis*	350 µg/mL	(Kvasnickova et al. 2015)
Baicalein	*C. krusei*	400 µg/mL	
Baicalein	*C. albicans*	1.28 mg/mL	(Liu et al. 2017)
Baicalein	*C. albicans* ATCC 10231	16 µg/mL	(Janeczko et al. 2022)
Baicalein	*C. albicans* SC5314	500 µg/mL	(Wang et al. 2015)

Flavonoid	*Candida* sp.	MIC value	Reference
Baicalein	*C. parapsilosis* ATCC 22019	250 µg/mL	
Baicalein	*C. albicans* ATCC 10231	6.4 µM	(Xin et al. 2019)
2′,4′-dihydroxy-5′-(1′′′,1′′′-dimethylallyl)-8-prenylpinocembrin (8PP)	*C. albicans*	150 µM	(Barceló et al. 2017)
Glabridin	*C. glabrata* 73	4-8 µg/mL	(Moazeni et al. 2017)
Dracorhodin perchlorate	*C. albicans*	64 µM	(Yang et al. 2018)
Epigallocatechin 3-O-gallate	*C. albicans* CDC 28304	7.81 µg/mL	(Behbehani et al. 2019)
Epigallocatechin 3-O-gallate	*C. dubliniensis* CDC 27963	15.63 µg/mL	
Quercetin	*C. albicans* 475	75 µg/mL	(Ivanov et al. 2020)
Quercetin	*C. albicans* ATCC 10231	> 83 µg/mL	(Salazar-Aranda et al. 2015)
Quercetin	*C. glabrata* 510	15.6 µg/mL	
Quercetin	*C. glabrata*	64 µg/mL	(Sadeghi-Ghadi et al. 2020)
Quercetin	*C. albicans* ATCC 10231	32 µg/mL	(Janeczko et al. 2022)
Quercetin	*C. krusei* ATCC 6258	2 µg/mL	(Rocha et al. 2019)
Quercetin	*C. parapsilosis sensu stricto*	0.5 µg/mL	
Quercetin	*C. albicans* ATCC 10231	512 µg/mL	(Gao et al. 2016)
Quercetin	*C. albicans* ATCC 10231	31.25 µg/mL	(Bodede et al. 2021)
Quercetin -3-O-methyl ether	*C. albicans* ATCC 10231	31.25 µg/mL	
Loureirin A	*C. albicans*	> 80 µg/mL	(Lin et al. 2019)
5,6,8-trihydroxy-7,4′ dimethoxy flavone	*C. albicans*	0.39 mg/mL	(Patel et al. 2020)
Kaempferol	*C. krusei* ATCC 6258	64 µg/mL	Rocha et al., 2019
Myricetin	*C. albicans*	32 µg/mL	(Dahibhate et al. 2021)
Myricitrin	*C. albicans* ATCC 10231	> 83 µg/mL	(Salazar-Aranda et al. 2015)
Myricitrin	*C. glabrata* 510	3.9 µg/mL	
Hesperetin	*C. albicans* SC5314	75 µM	(Hao et al. 2021)
Apigenin	*C. albicans* ATCC 90028	5 µg/mL	(Lee et al. 2018)
Luteolin	*C. glabrata* 493	3.9 µg/mL	(Salazar-Aranda et al. 2015)
Luteolin	*C. albicans* 475	37.5 µg/mL	(Ivanov et al. 2020)
Epigallocatechin gallate	*C. albicans* ATCC 2091	5 µg/mL	(Chen et al. 2015)
Epigallocatechin gallate	*C. glabrata* ATCC 2001	0.31 µg/mL	
Rutin	*C. albicans* 475	37.5 µg/mL	(Ivanov et al. 2020)

Flavonoid	*Candida* sp.	MIC value	Reference
Phloretin	*C. albicans* SC5314	74.55 µg/mL	(Liu et al. 2021)
Naringenin	*C. albicans* ATCC 10231	40 µg/mL	(Soberón et al. 2020)
Pinocembrin	*C. albicans* ATCC 10231	60 µg/mL	
5-hydroxy-7.4`-dimethoxyflavone	*C. albicans* ATCC 10231	45 µg/mL	(Mangoyi et al. 2015)
Epicatechin	*C. albicans* ATCC 10231	213 µg/mL	(Sachikonye and Mukanganyama 2016)
	C. krusei	88 µg/mL	
5, 7, 4´-trimethoxyflavone	*C. albicans* ATCC 90028	32 µg/mL	(de Oliveira Filho et al. 2016)
	C. albicans LM 86	32µg/mL	
2-phenyl-4H-chromen-4-one	*C. albicans* 33	> 62.5 µg/mL	(Simone et al. 2016)
	C. parapsilosis 2	31.25 µg/mL	
	C. glabrata 2	125 µg/mL	
Flavonoid derivatives			
Compound 4f and 4h	*C. albicans* 7535	1 µg/mL	(Wei et al. 2016)
Triacontyl *p*-coumarate	*C. krusei*	125 µg/mL	(Tamfu et al. 2020)
Triacontyl *p*-coumarate	*C. glabrata*	125 µg/mL	
Triacontyl *p*-coumarate	*C. albicans*	500 µg/mL	
Triacontyl *p*-coumarate	*C. parapsilosis*	500 µg/mL	
5n	*C. albicans*	0.0034 µM/mL	(Lal et al. 2018)
5o	*C. albicans*	0.0062 µM/mL	
3	*C. albicans*	15.62 µg/mL	(Burmaoglu et al. 2017)
3	*C. glabrata*	31.25 µg/mL	
3	*C. parapsilosis*	62.5 µg/mL	
10	*C. albicans*	125 µg/mL	
6,7,4`-O-triacetylscutellarein	*C. albicans*	> 1000 µg/mL	(Su et al. 2021)
3`-methoxy-hesperetin	*C. albicans* SC5314	50 µM	(Hao et al. 2021)
7-methoxy-hesperetin	*C. albicans* SC5314	25 µM	
7,3`-dimetoxy-hesperetin	*C. albicans* SC5314	75 µM	
Triazole chalcones	*C. albicans* ATCC 18804	> 500 µg/mL	(Santos et al. 2018)
4a	*C. albicans* ATCC 10231	125 µg/mL	(Illicachi et al. 2017)

Flavonoid	*Candida* sp.	MIC value	Reference
Chalcone 12	*C. albicans* ATCC 10231	15.6 µg/mL	(Andrade et al. 2018)
	C. glabrata ATCC 2001	15.6 µg/mL	
	C. krusei ATCC 34135	15.6 µg/mL	
	C. tropicalis ATCC 28707	15.6 µg/mL	
36	*C. albicans* IFO 0583	2 µg/mL	(El-Messery et al. 2018)
37	*C. albicans* IFO 0583	3 µg/mL	
38	*C. albicans* IFO 0583	3 µg/mL	
42	*C. albicans* IFO 0583	4 µg/mL	
2a	*C. albicans* ATCC 10231	6.4 µM	(Xin et al. 2019)
2d	*C. albicans* ATCC 10231	3.2 µM	
2e	*C. albicans* ATCC 10231	1.6 µM	
2f	*C. albicans* ATCC 10231	0.8 µM	
2g	*C. albicans* ATCC 10231	0.4 µM	
BrCl-flav	*C. albicans*	15.62 µg/mL	(Babii et al. 2021)
	C. parapsilosis		
	C. krusei		
	C. krusei ATCC 6258		
	C. glabrata		

2. The fungicidal activity of flavonoids

Minimum fungicidal concentration (MFC) is used to evaluate the fungicidal activity of flavonoids. The MFC is considered as the lowest concentration of an antifungal capable of killing ≥98–99.9% of viable cells as compared to the initial inoculum. In comparison with MIC assessment, the parameters used in practice for MFC determination are not fully standardized because of the results variations. Due to the lack of reference tests, the MFC values are frequently determined using macro- and microdilution methods. However, the values determined for the same antifungal agent varies depending on the methods employed, as shown by Pujol et al. who found that higher MFC values were recorded for amphotericin B when macrodilution method was used compared to the microdilution method (Pujol et al. 2000).

MFC values could be a useful clinical tool for the treatment of severe fungal infections, especially in immunocompromised patients (Pujol et al. 2000). However, the importance of MIC values determined *in vitro* is not yet validated in clinical trials (Espinel-Ingroff et al. 2002). Therefore, the practical importance of MFC in clinical therapies is yet to be evaluated by further studies (Arikan 2007).

The fungicidal activity of flavonoids in terms of MFC is less documented in literature compared to MIC due to results variations and the lack of reference tests. The MFC values of some flavonoids that have been reported from 2016 to date are available in Table 2.

Many flavonoids were described in literature as fungicidal agents. Thus, Behbehani et al. presented the important anti-*Candida* activity of flavonoid epigallocatechin 3-O-gallate, with recorded MFC values 2-3 times higher compared to MIC. The flavonoid tested against *C. glabrata* CDC 28398 reduced the number of viable cells with 99.9% at a concentration equal to15.63 µg/mL. Close MFC values (31.25 µg/mL) were evidenced when the flavonoid was tested against *C. dubliniensis* CDC 27963 and *C. albicans* CDC 28304 strains (Behbehani et al. 2019).

A similar fungicidal activity was reported for chalcone 12 against *C. albicans* ATCC 10231 and *C. krusei* ATCC 34135 strains, with MFC of 15.6 µg/mL. Tested on *C. tropicalis* ATCC 28707 and *C. glabrata* ATCC 2001, chalcone 12 also showed important anti-*Candida* activity, with MFC values of 31.25 µg/mL and 125 µg/mL, respectively (Andrade et al. 2018).

In the study of Ivanov *et al*., flavonoids luteolin, quercitrin, isoquercetin and rutin were found to have a MFC value of 75 µg/mL against *C. albicans* 475 strain (Ivanov et al. 2020).
De Oliveira Filho et al. showed that 5, 7, 4′-trimethoxyflavone isolated from *Praxelis clematidea* has MFC values of 64 µg/mL against *C. albicans* ATCC 90028 and 1024 µg/mL against *C. albicans* LM 86 (de Oliveira Filho et al. 2016).
A lower fungicidal activity was reported for 5,6,8-trihydroxy-7,4′ dimethoxy flavone isolated from *Dodonaea viscosa* var. *angustifolia* against *C. albicans* cells, with a MFC value of 1.56 mg/mL (Patel et al. 2020).
An important fungicidal activity has also been reported by Babii et al. for BrCl flavonoid. After 12 h of incubation in the presence of the synthetic flavonoid (31.25 µg/mL), no viable cells were detected, a total kill effect being evidenced against two fluconazole resistant isolates of *C. albicans* and *C. krusei* (Babii et al. 2021).
As we have shown so far, flavonoids are compounds that display amazing anti-*Candida* properties, similarly to the conventional antifungals used in clinical therapy. However, caution is advised when comparing the antifungal properties of the same flavonoids or flavonoids belonging to the same classes due to the lack of standardized methods of analysis which leads to important variations of the results. Even if the methods are standardized, as happens in the case of the MIC determination, different experimental conditions could also be real challenges when it comes to comparing the antifungal activity of flavonoids. Thus, the use of different *Candida* strains, diverse culture media, various incubation conditions (temperature, time, etc.), reading the results employing different systems are factors which significantly influence the consistency of results.

Table 2. Fungicidal potential of reported flavonoids against *Candida* species

Flavonoid	***Candida* sp.**	**MFC**	**Reference**
Epigallocatechin 3-O-gallate	*C. dubliniensis* CDC 27963	31.25 µg/mL	(Behbehani et al. 2019)
	C. albicans CDC 28304	31.25 µg/mL	
	C. glabrata CDC 28398	15.63 µg/mL	
Baicalein	*C. albicans*	20.48 mg/mL	(Liu et al. 2017)
Baicalein	*C. albicans* ATCC 10231	> 64 µg/mL	(Janeczko et al. 2022)
Quercetin	*C. albicans* ATCC 10231	> 128 µg/mL	
5,6,8-trihydroxy-7,4′ dimethoxy flavone	*C. albicans*	1.56 mg/mL	(Patel et al. 2020)
5, 7, 4′-trimethoxyflavone	*C. albicans* ATCC 90028	64 µg/mL	(de Oliveira Filho et al. 2016)
	C. albicans LM 86	1024 µg/mL	
Luteolin	*C. albicans* 475	75 µg/mL	(Ivanov et al. 2020)
Quercetin	*C. albicans* 475	150 µg/mL	
Quercitrin	*C. albicans* 475	75 µg/mL	
Isoquercetin	*C. albicans* 475	75 µg/mL	
Rutin	*C. albicans* 475	75 µg/mL	
Chalcone 12	*C. albicans* ATCC 10231	15.6 µg/mL	(Andrade et al. 2018)
	C. glabrata ATCC 2001	125 µg/mL	
	C. krusei ATCC 34135	15.6 µg/mL	
	C. tropicalis ATCC 28707	31.25 µg/mL	

Flavonoid	*Candida* sp.	MFC	Reference
2-phenyl-4H-chromen-4-one	*C. albicans* 33	> 250 µg/mL	(Simone et al. 2016)
	C. parapsilosis 2	31.25 µg/mL	
	C. glabrata 2	125 µg/mL	
BrCl-flav	*C. albicans*	31.25 µg/mL	(Babii et al. 2021)
	C. parapsilosis		
	C. krusei		
	C. krusei ATCC 6258		
	C. glabrata		

3. Mechanism of action

The fungistatic and fungicidal properties of flavonoids shown so far explain the large use of these compounds as antimicrobial agents in traditional medicine. Antifungal activity is related to different mechanisms of action. Thus, flavonoids can target different cell structures such as plasma membrane, cell wall, DNA, or mitochondria. Also, flavonoids may interfere with protein synthesis and cell division (Al Aboody and Mickymaray 2020).

The cell membrane acts as a barrier relative impermeable to fungal cells (Ma et al. 2020) and plays a critical role in promoting *Candida* cell virulence (Douglas and Konopka 2016). The plasma membrane is also involved in the excretion of virulence factors, the synthesis of cell wall and endocytosis (Douglas and Konopka 2016). Due to its critical role, the cell membrane has become the target of the most used antifungal drugs (Douglas and Konopka 2016).

Papyriflavonol A, a prenylated flavonol that has been isolated from the root bark of *Broussonetia papyrifera*, was reported as an anticandidal compound. Sohn showed that the papyriflavonol A potential is explained by its capability to disrupt the integrity of the cell membrane (Sohn 2010).

A similar mechanism of action was reported for 5,6,8-trihydroxy-7,4′ dimethoxy flavone (TMMC) isolated from *Dodonaea viscosa* var. *angustifolia*. At lower concentrations, corresponding to 0.5 × MIC and MIC, the compound affected the integrity of the plasma membrane, allowing a slight diffusion of propidium iodide into cells. The exposure to higher concentrations led to the complete destruction of the plasma membrane, proven by the visualization of an increased number of fluorescent cells (Patel et al. 2020).

The plasmatic membrane of *Candida* cells exposed to epigallocatechin 3-O-gallate (EGCG) for 12 hours became permeable to propidium iodide and showed deformations compared to control cells which remain intact. This indicates that activity of EGCG on *Candida* cells could be explained by damaging the cell membrane (Behbehani et al. 2019).

The impairment of *C. albicans* cells membrane after only 2.5 hours of incubation in the presence of myricetin (20 µg/mL), followed by a visible lysis after 4 hours was recorded by Lee and Kim. In addition, cell debris, characteristic to damaged cell wall, were observed in myricetin-treated *C. albicans* cells (Lee and Kim 2022).

An increased fluorescence of *C. albicans* cells incubated in the presence of apigenin was observed by Lee et al., indicating severe disruption of cell membrane (Lee et al. 2018).

The antifungal activity of isoquercitrin was related to its ability to inhibit fatty acid synthase and to disrupt the plasma membrane of *C. albicans* and *C. parapsilosis* (Yun et al. 2015). A similar effect was reported by da Silva et al. for catechin against *C. tropicalis* cells. At concentration of 16 µg/mL, catechin inhibited fatty acid synthase by activation of phosphatidylserine, also inducing ROS intracellular accumulation, mitochondrial depolarization and DNA fragmentation (da Silva et al. 2014). Catechin was also found responsible for the inhibition of nucleic acid synthesis, being proposed as a potential antifungal candidate in clinical therapy of candidiasis (Saito et al. 2013, Aboody and Mickymary 2020).

Baicalein, isolated from *Scutellaria baicalensis*, exerted anti-*C. albicans* activity by inducing deformation of cell membrane structure and the efflux of intracellular compounds (Da et al. 2019). Also, baicalein was responsible for mitochondrial membrane impairment and increased intracellular ROS levels, leading to apoptosis (Dai et al. 2009). Inconsistent data about baicalein mechanism of action are reported by Kang et al. According to the authors, the antifungal activity of the flavonoid against C. *krusei* strains is related to mitochondria homeostasis impairment and not to intracellular ROS generation or apoptosis (Kang et al. 2010).
Contradictory data of Wang et al. are suggesting that baicalin mechanism of action is not membrane related. The authors reported that in the presence of baicalin (1000 μg/mL), the membrane of *C. albicans* SC5314 cells was not affected, only 7.91% of the cells being fluorescent due to propidium iodide uptake (Wang et al. 2015).
Protein and RNA synthesis in *C. albicans* cells was impaired by compounds such as gallic acid (isolated from *Paeonia rockii*) or gallotannin (extracted from *Syzygium cordatum*) (Picerno et al. 2011, Mulaudzi et al. 2012).
In combination with miconazole, 5-hydroxy-7,4′-dimethoxyflavone decreased ergosterol and inhibited drug efflux pumps in *C. albicans* cells (Mangoyi et al. 2015). Similarly, sedonan A isolated from *Dalea formosa* inhibited efflux pumps in *C. albicans* and *C. glabrata*, interfering with different intracellular transcription genes (Belofsky et al. 2013).
Glabridin, an isoflavan isolated from *Glycyrrhiza glabra*, showed important synergistic effect with fluconazole against resistant strains of *C. albicans* and *C. tropicalis*, with an increased membrane permeability, DNA damage an induced apoptosis (Liu et al. 2014, Moazeni et al. 2017).
Curcumin, quercetin and resveratrol were found to modulate the activity of transcription factors which control mitochondrial proteins' expression (Oliveira et al. 2016, Aboody and Mickymaray 2020). Same compound poses pro-apoptotic properties by discharge of cytochrome c from mitochondria, or by upregulating pro-apoptotic proteins and downregulating anti-apoptotic proteins (Gibellini et al. 2015). In combination with fluconazole and amphotericin B, curcumin showed synergistic interaction against *C. albicans* by interfering with the expression of genes related to fungal oxidative stress, such as superoxide dismutase, catalase, and oxidoreductase (Sharma and Prasad 2011, Aboody and Mickymaray 2020).
Synthetic flavonoids, such as 2′4′-dihydroxychalcone, showed anti-*Candida* activity based on their capacity to interfere with ergosterol and cellular membrane (Andrade et al. 2018).
A chalcone derivative synthetized by Łącka et al. showed important antifungal activity against fluconazole resistant *Candida* strains, targeting fungal cell wall formation and cellular division (Łącka et al. 2011).
Azole and non-azole derivatives with important activity against resistant *C. albicans* isolates affected the cell membrane by decreasing ergosterol biosynthesis (Ahmad et al. 2017). A similar effect was also recorded for an imidazole–chalcone derivative (3c), which decreased the level of ergosterol in *C. krusei* cells (Osmaniye et al. 2018).
Synthetic tricyclic sulfur containing flavonoid BrCl showed important anticandidal activity, targeting the cell membrane and not the cell wall. Tested against clinical isolates of *C. albicans*, *C. parapsilosis*, *C. krusei* and *C. glabrata*, BrCl-flav inhibited fungal growth. The integrity of membrane structure was significantly affected, inducing cell lysis, as revealed by fluorescence microscopy and SEM (Babii et al. 2021).
As it has been shown so far, flavonoids present multiple mechanisms of action, targeting structures and cellular processes essential for the survival of *Candida* cells, explaining the increased antifungal efficiency.

4. Flavonoids as anti-virulence agents

Antimicrobial resistance (AMR) occurs when microorganisms, including fungi, are no longer responding to conventional drugs due to multiple cellular changes and adaptations. Therefore, the infections caused by antimicrobial resistant strains are harder to treat, leading to severe illness and death. Considering the enormous medical, social and economic impact, WHO declared in 2018 AMR a global health and economic threat.

Several innovative therapeutic strategies were proposed to fight AMR. Anti-virulence is considered one of the most promising alternatives for effective control of resistant pathogens. Anti-virulence agents target different microbial virulence cellular factors used by pathogenic microorganisms to invade and colonize the host (Fleitas Martínez et al. 2019).

Hyphal formation is a well-known virulence factor of *Candida albicans*, responsible for the initiation of infection by colonization of host tissular structures. *C. albicans* can shift its morphology from round yeast cells to filamentous hyphae (Yoo et al. 2020). This transition leads to the appearance of pathogenic forms (Talapko et al. 2021). The phenotypic switch allows this organism to invade the host and facilitate the disease (Deepa et al. 2015, Yoo et al. 2020, Mendoza-Reyes et al. 2022).

Many flavonoids exhibited anti-virulence activity, inhibiting yeast to hyphal transition for different *C. albicans* strains. Licochalcone A inhibited *C. albicans* LAM-1 and *C. albicans* ATCC 28366 hyphal formation for 2 and 4 hours at concentrations of 100 µg/mL and 200 µg/mL. Incubation of *C. albicans* LAM-1 in the presence of licochalcone A (100 µg/mL) resulted in a 79% inhibition of hyphae formation after only 2 hours and 82% inhibition after 4 hours. Against *C. albicans* ATCC 28366, licochalcone A completely prevented hyphae formation (Messier and Grenier 2011).

Singh et al. reported that quercetin completely blocked *C. albicans* ATCC 28366 hyphal formation at a concentration of 200 µg/mL (Singh et al. 2015). Dracorhodin perchlorate, a derivative of the flavonoid dracohordin, inhibited the morphological transition of *C. albicans* cells. After exposure to dracohordin, the number of hyphae and their length decreased compared to control. However, the ability to inhibit *C. albicans* hyphae varied depending on the culturing conditions (Yang et al. 2018).

Flavonoids effects against hyphal formation may vary depending on the concentration used. Thus, Lin et al. showed that at 20 µg/mL loureirin A inhibited the transition from yeast to hyphae, and at 40 µg/mL completely disrupted formed hyphae. The authors evidenced that the loureirin A is capable of hyphal inhibition by reducing the expression of some genes involved in hyphal elongation, such as *ece1* and *hwp1* (Lin et al. 2019).

Abirami et al. reported that morin treated *C. albicans* ATCC 90028 cells (150 µg/mL) formed a reduced number of small hyphae, while the untreated control was capable of normal hyphal growth (Abirami et al. 2020).

Phloretin at concentrations equivalent to 1 × MIC and 2 × MIC significantly inhibited the hyphae formation of *C. albicans* by 70%. The yeast to hyphal formation process was affected by phloretin at molecular level. Thus, the relative expression of the genes involved in hyphae elongation (*eap1*, *ece1*, *hwp1* and *als3*) was significantly reduced by phloretin (Liu et al. 2021).

Janeczko et al. showed that both baicalein and quercetin were able to repress the formation of hyphae by two *C. albicans* clinical isolates. Only sporadic hyphae were evidenced when cells were treated with concentration corresponding to ⅛ MIC (8 µg/mL) (Janeczko et al. 2022).

Our previous studies concerning the anticandidal activity of synthetic flavonoid BrCl-flav revealed that the compound prevented the transition of *C. albicans* from yeast to hyphae, after 6 h of incubation. In the presence of BrCl-flav at concentration equivalent to 2 × MIC (31.24 µg/mL), the exposed cells did not form hyphae up to 48 h. Moreover, SEM images exposed irreversible morphological damage of BrCl-flav treated fungal cells.

Yeast to hyphae transition also initiate the biofilm formation, contributing to *Candida* pathogenesis (Yang et al. 2018). The capability of *Candida* sp. to form biofilms on different

surfaces such as living tissue or medical devices like catheters, prosthetic heart valves and joint replacements (Cavalheiro and Teixeira 2018) is considered as an important virulence factor (Peralta et al. 2015, Gulati and Nobile 2016, Tsui et al. 2016). Biofilms produced by *Candida albicans* are three-dimensional structures consisting of several cell types (yeast, hyphae and pseudohyphae) set in a matrix of extracellular polysaccharides (Gulati and Nobile 2016, Cavalheiro and Teixeira 2018, Pereira et al. 2021). Inside this matrix, individual cells present specific metabolic features, being more resistant to antifungal drugs or host immune. Therefore, *Candida* infections related to biofilm formation are difficult to treat, recurrent and life threatening.

Many studies reported the anti-biofilm activity of different flavonoids against *Candida* species. Fu et al. found that luteolin at a concentration equivalent to ½ MIC (16 µg/mL) reduced *C. albicans* SC5314 cell adhesion by 63%. In addition, luteolin caused significant decreases in *C. albicans* biofilm biomass by 62 and 51.7%. Also, luteolin-treated biofilms visualized using microscopy techniques appeared to be sparse and thinner compared to control (Fu et al. 2021). Manoharan et al. showed that chrysazin and alizarin inhibited both biofilm development as well as hyphal formation by *C. albicans* (Manoharan et al. 2017).

Lobo et al. demonstrated that 4`-hydroxychalcones eradicated preformed *C. albicans* SC5314 biofilms at concentrations between 125 and 1250 µg/mL. Also, a reduction in CFU/mL of 4 logs and 5 logs was recorded compared with the control (Lobo et al. 2021). Gabriela et al., also reported that 2`,4`-dihydroxychalcone, isolated from *Zuccagnia punctata* was able to impede *C. albicans* biofilm formation (Gabriela et al. 2014). Also, licochalcone-A at a concentration of 625 µM exhibited significant anti-biofilm activity against *C. albicans* (Seleem et al. 2016).

Loureirin A, one of the main active molecules of *Draconis sanguis*, showed an important anti-*Candida* biofilm activity. Lin et al. observed that loureirin A inhibited the formation of biofilms in a dose-dependent manner. *C. albicans* biofilm formation was inhibited by 37% in the presence of 5 µg/mL loureirin A, while at a concentration of 80 µg/mL the biofilm was inhibited by 70% (Lin et al. 2019).

At concentrations between 4 µg/mL and 32 µg/mL, baicalein inhibited *C. albicans* biofilms in a dose dependent manner. The main mechanism involved is related to the reduction of mRNA expression and cell surface hydrophobicity (Cao et al. 2008).

A concentration-dependent anti-*Candida* biofilm activity has also been observed for quercetin. At a concentration of 200 µg/mL, quercetin almost completely inhibited *Candida albicans* NBC099 biofilm formation, and at a concentration of 100 µg/mL, the biofilm was inhibited by 80% (Singh et al. 2015).

Morin used at a concentration of 150 µg/mL exhibited a maximum inhibition of the *C. albicans* biofilm by 93% (Abirami et al. 2020).

Liu et al. investigated the effect of phloretin against *C. albicans* biofilm formation and found that the percentage of inhibition was dose dependent. The results suggest that the metabolic activity of the *C. albicans* biofilm was reduced in the presence of phloretin at concentrations of 37.28 µg/mL (0.5 × MIC), 74.55 µg/mL (MIC) and 149.1 µg/mL (2 × MIC), in correlation with a significant reduction of biofilm biomass (Liu et al. 2021).

Anti-biofilm activity has been reported for isoquercetin and apigenin against *C. albicans*. The most promising inhibition (76%) was observed by Ivanov et al. for quercetin at a concentration corresponding to MIC (37.5 µg/mL), while at a concentration equal to ½ MIC (18.75 µg/mL), both quercetin and apigenin were able to prevent biofilm formation by more than 60% (Ivanov et al. 2020). Lee et al. reported an important activity against *C. albicans* ATCC 90028 biofilm for apigenin isolated from *Aster yomena*. The metabolic activity of mature biofilm decreased by 31.8% in the presence of apigenin at a concentration of 5 µg/mL (Lee et al. 2018).

Prenylflavanone 8PP, extracted from *Dalea elegans*, exhibited anti-biofilm activity against sensitive and azole-resistant *C. albicans* strains at 100 µM by elevating ROS and reactive nitrogen intermediates levels (Peralta et al. 2015).
Rocha et al. investigated the activity of kaempferol on biofilm produced by *Candida parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis*. This flavonoid caused a reduction in the metabolic activity of *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis* biofilms and decreased the biomass at concentrations equal to the MIC (32 µg/mL, 64 µg/mL, and 32 µg/mL) (Rocha et al. 2019).
The flavone 5,6,8-trihydroxy-7,4` dimethoxy flavone (5,6,8-trihydroxy-7-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one) - TMMC was reported to be active against biofilm formation and mature *C. albicans* biofilms. The result obtained by Patel et al. showed that the effect of the TMMC is dose dependent. TMMC reduced biofilm formation by 77.2% and destroyed mature biofilm by 28.2% at a concentration equal to the MIC (0.39 mg/mL) (Patel et al. 2020).
Synergistic combinations between flavonoids and conventional antifungals were reported to exhibit important anti-biofilm activities against different *Candida* species. Epigallocatechin gallate in combination with amphotericin B, fluconazole and miconazole inhibited biofilms of different *Candida* species (Ning et al. 2015). Similar synergistic anti-biofilm effects were reported by Gao et al. Thus, quercetin in combination with fluconazole inhibited *C. albicans* biofilm by disturbing the expression of biofilm formation related genes (Gao et al. 2016). Quercetin in combination with kaempferol (450 µg/mL) and a HDAC inhibitor sodium butyrate (30 mM) inhibited *C. tropicalis* biofilms (Rajasekharan et al. 2015).
Synthetic flavonoid BrCl-flav investigated by our research group impeded *C. albicans, C. krusei* biofilm formation, the effect being dose-dependent. Significant reduction of biofilm formation was recorded at both inhibitory and subinhibitory concentrations (¼ × MIC and ½ × MIC) (Babii et al. 2021).

Conclusions

The increase of infections caused by pathogenic *Candida* species worldwide is alarming. Moreover, the appearance of multidrug-resistant strains impedes candidiasis treatment. Flavonoids, used in traditional medicine for their biological properties, can constitute an alternative solution for the treatment of *Candida* infections. Numerous studies highlighted the fungistatic and fungicidal properties of flavonoids against species such as *C. albicans*, *C. krusei*, *C. tropicalis* or *C. parapsilosis*. The main mechanism of action is most frequently related to cell membrane damage. Thus, natural flavonoids such as apigenin, myricetin, baicalein or papyriflavonol A and synthetic flavonoids such as 2′4′-dihydroxychalcone, chalcone derivatives or sulfur containing flavonoid BrCl target the cell membrane, inducing deformation, increased permeability and integrity disruption. Both natural and synthetic flavonoids pose important anti-virulence and antibiofilm activities, offering great hopes for the development of effective anti-*Candida* therapies in the future.

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