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Flavonoid as Anti-Candida Agents

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Abstract

Vulvovaginal candidiasis is the second most common cause of vaginitis after bacterial vaginosis with at least 75 – 80% of women have had one episode of VVC in their lifetime. Their prevalent resistance to most commonly used antifungal agents makes their treatment a challenge to physicians. Flavonoids have been shown to possess potent anti-*Candida* properties which can inhibit the growth and proliferation of *Candida* species through various mechanisms, including inhibition of fungal cell wall synthesis, disruption of fungal cell membrane integrity, and interference with fungal cell signaling pathways. Their potency makes them potential candidates for the development of antifungal agents for the treatment of candidiasis, alone or in combination with existing antifungal drugs. The review aims to explore the mechanisms by which flavonoids inhibit the growth and proliferation of *Candida* species, including the inhibition of fungal cell wall synthesis, disruption of fungal cell membrane integrity, and interference with fungal cell signaling pathways.

Keywords: flavonoid, Anti-Candida Agents

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INTRODUCTION

Flavonoids are a group of natural compounds that belong to the family of polyphenols, characterized by their chemical structure, which consists of two benzene rings connected by a three-carbon chain [1]. Flavonoids are commonly found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine [2-3]. They are known for their antioxidant properties and have been associated with various health benefits, including reducing the risk of chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders [4]. There are over 6,000 different types of flavonoids, and they are divided into subclasses based on their chemical structure and the presence or absence of certain functional groups [5-6]. Based on the chemical structure of their carbon rings, flavonoids are divided into seven different subclasses: chalcones, flavones, isoflavones, flavanols, flavanones, flavonols, and anthocyanidins [1-2, 5]. Vulvovaginal candidiasis (VVC) is a very common mucosal infection of female reproductive tract caused by polymorphic opportunistic fungi of *Candida* species, most commonly by *Candida albicans* [7–9]. Vulvovaginal candidiasis is the second most common cause of vaginitis after anaerobic bacterial vaginosis. At least 75 – 80% of women have had one episode of VVC in their lifetime, and 9% of them experienced more than three episodes in one year, called recurrent vulvovaginal candidiasis [10]. In Indonesia, recurrent vulvovaginal candidiasis affects more than 5 million women each year with a prevalence annual 4184 per 100,000 women [11]. Flavonoids have been shown to possess potent anti-*Candida* properties, meaning they can inhibit the growth and proliferation of Candida species [12-13]. Derivates of flavonoids such as luteolin, quercitrin, isoquercitrin, and rutin exhibited inhibitory effects on the growth of *Candida albicans*, with a minimal inhibitory concentration of 37.5 µg/mL. Additionally, the application of isoquercitrin resulted in a significant reduction of *C. albicans* biofilm formation by 76% and inhibited hyphal formation by the yeast [14]. The antifungal activity of flavonoids against *Candida* species is shown through various mechanisms, including inhibition of fungal cell wall synthesis, disruption of fungal cell membrane integrity, and interference with fungal cell signaling pathways. Various flavonoids have been extracted and investigated for their antifungal activity, suggesting that they may have potential as efficient and cost-effective therapies for candidiasis [1, 5, 15]. Hussain et

al. has shown the potency of quercetin, catechine, and hydroxybenzyltaxifolin isolated from mango (*Mangifera indica* L.) leaves in suppressing fungal growth [16]. Guo et al. proved the antifungal activity of citrus' extract of flavones by inhibiting spore germination and mycelial growth [17], and studies by Simonetti et al. stated that extracts from *Vitis vinifera* L., specifically quercetin and catechin, exhibit antifungal properties against *Candida albicans* [18]. Overall, the anti-*Candida* properties of flavonoids make them potential candidates for the development of natural antifungal agents for the treatment of candidiasis, either alone or in combination with existing antifungal drugs [19-20]. This review summarizes mechanism of flavonoid in inhibiting *Candida* growth, including fungal cell wall synthesis inhibition, disruption of cell membrane integrity, and interference with cell signaling pathways, along with its potential as antifungal agents against *Candida albicans*.

Basic Structure and Type of Flavonoid

Flavonoids, belonging to the family of polyphenols, are a group of natural compounds characterized by their chemical structure, which consists of two benzene rings connected by a three-carbon chain. Commonly found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine, flavonoids are widely distributed in nature [2-3]. The basic structure of flavonoids is a diphenylpropane (C6-C3-C6) skeleton, consisting of two aromatic rings (A and B) joined by a three-carbon chain (C). The rings are denoted as A and B, and the carbons of the C chain are numbered 2', 3' and 4' to differentiate them from the carbons in the A and B rings. The A ring contains six carbon atoms and one oxygen atom, while the B ring contains six carbon atoms. The C chain connects the two rings and consists of three carbon atoms. The flavonoid structure can be modified by the addition of hydroxyl (-OH) and other functional groups, which can alter its physical and chemical properties [21]. Flavonoids can be classified into several subgroups based on the position and nature of these functional groups. Some of the most common types of flavonoids shows on **Figure** 1 include: flavones, flavonols, flavanones, flavan-3-ols, chalcones, isoflavones, and anthocyanidins [1-2]. These different types of flavonoids can have varying health benefits and are found in a wide range of plant-based foods [22]. The diversity in structure and properties of flavonoids makes them an important group of natural compounds with a range of potential health benefits [1]. Among them, flavonols and chalcones especially have shown the most significant antifungal activities [2]. In their study, Evensen et al. investigated the antifungal properties of major polyphenol compounds found in green tea, specifically epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), and epicatechin-3-gallate (ECG), extracted from *Camellia sinensis*. The in vitro analysis showed that treatment with 1.0 mmol/L EGCG resulted in a significant reduction of biofilm formation of *Candida albicans* by 75% in viable cells [23]. Loureirin A, a component derived from chalcones, has been shown to possess effective antifungal properties, as demonstrated in two different animal models [24-25]. Its use has also been associated with the suppression of pathogenic traits [25].

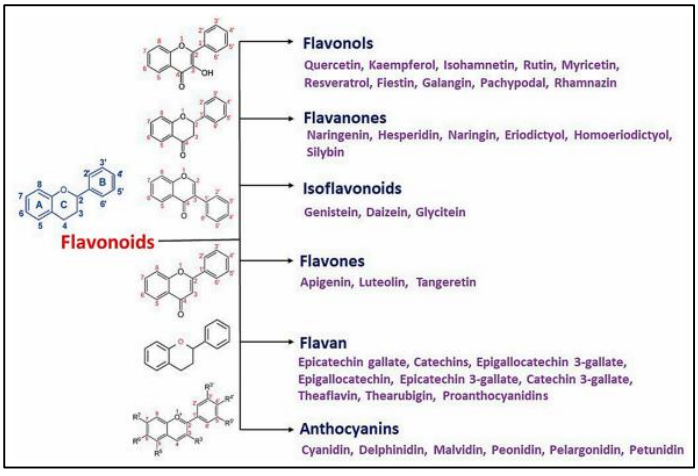

Figure 1. Structure of various subclasses of flavonoids [1]

Biosynthesis and Pathways of Flavonoids

Flavonoids, a diverse class of secondary metabolites, are synthesized through the phenylpropanoid pathway, which involves several enzymatic steps [26-27]. The genetic perspective of flavonoid biosynthesis has been extensively studied, with significant progress made in understanding the

pathway using various plant species as experimental models [27-28]. Biosynthesis of flavonoids encompasses two primary pathways: the acetate pathway, which involves ring A, and the shikimate pathway, which involves ring B and the linking chain (ring C). The combination of rings A and B results in the formation of chalcone, which is then transformed into flavanone through cyclization facilitated by isomerases. Flavanone serves as the initial building block for the synthesis of diverse flavonoids. The structural variation observed among flavonoids is attributed to the varying levels of unsaturation and oxidation in ring C (**Figure** 2). Flavonoids encompass several categories, including flavanones, flavanols, flavones, and flavonols, and there are additional classes such as isoflavones, biflavonoids, and chalcones [26].

The biosynthesis of flavonoids initiates with the conversion of phenylalanine into 4-coumaroyl-CoA, an essential precursor in the pathway. Chalcone synthase (CHS) is the first enzyme dedicated to the flavonoid pathway, and it produces chalcone scaffolds that serve as the foundation for the synthesis of various subclasses of flavonoids. Through the involvement of enzymes such as isomerases, reductases, hydroxylases, and dioxygenases, the basic flavonoid structure undergoes further modifications, resulting in the production of different flavonoid compounds. Subsequently, transferases come into play and add sugars, methyl groups, and acyl moieties to the flavonoid backbone, influencing their solubility, reactivity, and interaction with specific targets within cells [28].

Flavonoid O-xyloside and flavonoid O-arabinoside are two types of flavonoid O-pentoses produced by plants. However, studying their biological properties is challenging due to the limited natural production of flavonoids [29]. To harness their biological potential and obtain enantiomerically pure forms, numerous chemical methodologies have been developed to acquire nature-inspired flavonoids. Stereoselective synthesis has emerged as a valuable tool for obtaining compounds with both biological relevance and high enantiomeric purity. These approaches include stereoselective chalcone epoxidation, sharpless asymmetric dihydroxylation, Mitsunobu reaction, and the cycloaddition of 1,4-benzoquinone. Additionally, techniques such as the use of chiral auxiliaries, organo- and organometallic reactions, bio-catalysis, and the chiral pool approach have been employed to obtain chiral bioactive flavonoids with a high enantiomeric ratio [30].

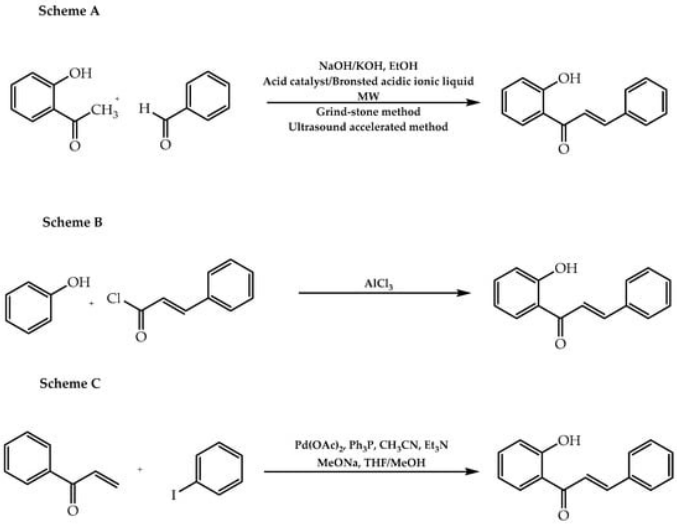

Figure 2. Synthetic approaches for 2′-hidroxychalcones [30].

In natural systems, 2'-hydroxychalcones serve as intermediates for the synthesis of various classes of flavonoids, including flavonols, flavones, and flavanones. Synthetic routes have been developed to obtain these intermediates, employing methods such as the Claisen-Schmidt reaction, Friedel-Crafts condensation, and Heck coupling. In the Claisen-Schmidt reaction, an aromatic aldehyde and a substituted acetophenone react under basic catalysis, resulting in the formation of 2'-hydroxychalcones. Microwave and ultrasound techniques have been utilized to enhance the yields and reduce reaction times. The Friedel-Crafts method involves the condensation of (E)-3-phenylprop-2-enoyl chloride with phenols, using AlCl3 as a catalyst to generate 2'-hydroxychalcones. Another approach, the Heck coupling pathway, combines aryl α,β-unsaturated ketones with iodobenzene to yield the desired chalcone product. These synthetic methodologies provide efficient routes for the synthesis of 2'-hydroxychalcones, enabling further exploration of their potential biological activities and their utilization

as building blocks for the production of diverse flavonoid compounds [30].

Candidal Infection: Biology and Pathogenicity

Candida is a unicellular structure, often called yeast or yeast-like, with over 150 species known, though only 17 known to infect humans. The common species that cause vulvovaginitis are *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. stellatoidea*, and *C. parapsilosis* [9-12]. In healthy individuals, C. albicans commonly resides in the oral, vaginal, and gastrointestinal mucosa as a harmless commensal. However, under certain conditions, such as disturbances in the local microbiota, weakening of normal tissue barriers, or compromised immune defenses, this fungus has the potential to cause infections [31].

Candida albicans, the most commonly isolated fungal pathogen in humans, is responsible for around 50% of all *Candida* infections, with the remaining cases being caused by less pathogenic non-albicans Candida species (NACS) [32].

Candida albicans has several morphologies (blastospores, pseudohyphae, and hyphae) [33]. Hyphae are elongated structures found in filamentous fungi and have a thread-like appearance. Pseudohyphae, on the other hand, are chains of newly divided cells observed in unicellular fungi. The key distinction between hyphae and pseudohyphae lies in their mode of formation. In the case of Candida albicans, a diploid yeast-like fungus, cultures of this species produce large, thick-walled chlamydoconidia either at the tips of true hyphae or along the pseudohyphae [34]. Blastospores divide asexually by budding, which is the growth of new cell material on the surface of the blastospore [35]. The cell wall of *C. albicans* contains carbohydrates and proteins which are not found in the human body, therefore making them an ideal immunological target. Consequently, most of these fungal PAMPs (pathogen-associated molecular patterns) that activate and modulate the immune response are cell wall components [36].

In human microbiome, *C. albicans* is present in the form of blastospores. The transition from the blastospore to the hyphal form is the transition to the pathogenic form. The hyphal form is invasive, and in this form, cells enter host tissue by active penetration and induce endocytosis. Endocytosis is mediated by hyphal invasion and depends on host activity whereas active penetration depends on fungal activity (**Figure** 3). Several signaling pathways are involved in hyphal formation. The most important is cAMP-dependent protein kinase A (cyclic adenosine monophosphate PKA) [35, 37].

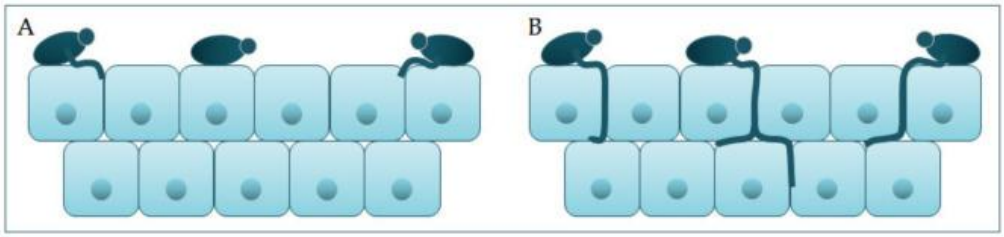

Figure 3. Schematic presentation of (A) adhesion and colonization; (B) penetration and invasion of *C. albicans* [35]

Candida albicans infects cells directly through hyphal invasion or indirectly through contact between PAMPs (Pathogen-associated Molecular Factors) and receptors called PRRs (Pattern Recognition Receptors). This binding will trigger inflammatory immune mediators, such as chemokines, cytokines, antimicrobial peptides, to be secreted and recruit innate immune cells, such as macrophages, dendritic cells, and neutrophils (**Figure** 4). These cells also recognize PAMPs via PRR on their surface, bind to pathogens, and stimulate Candida elimination via phagocytosis [38-39].

Another significant characteristic of *C. albicans* that contributes to its virulence is its ability to form biofilms on various surfaces. Biofilms are complex communities of microorganisms that attach to surfaces and form a protective matrix of extracellular polymeric substances (EPS) that shields them from environmental stresses, such as host immune responses and antimicrobial agents. The biofilm of *Candida albicans* consists of yeast cells, hyphae (long filamentous structures), and extracellular matrix material, including polysaccharides, proteins, and lipids. The matrix provides protection and nutrients to the cells within the biofilm, as well as a surface for attachment to host tissues [35]. The formation of biofilms occurs through a series of steps, starting with the adherence of yeast cells to the substrate, followed by their proliferation and the development of hyphal cells in the upper part of the biofilm. As the biofilm matures, extracellular matrix material accumulates, and eventually, yeast cells disperse from the complex structure. Mature biofilms exhibit increased resistance to antimicrobial

agents and host immune factors compared to freely suspended (planktonic) cells [40].

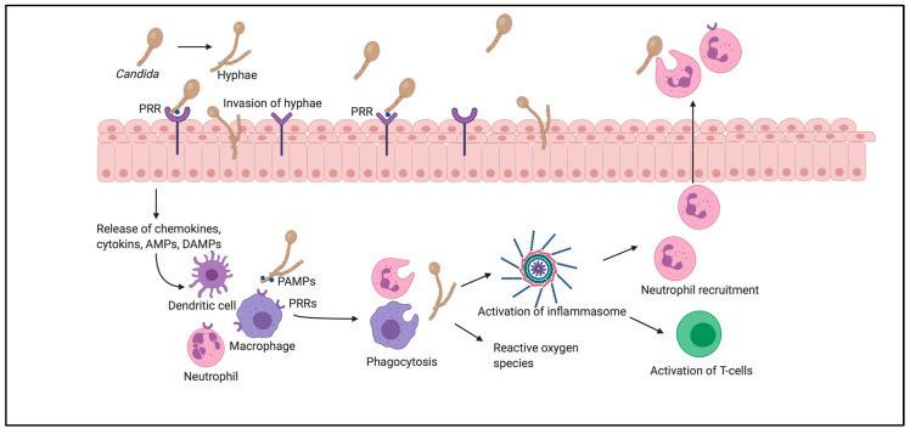

Figure 4. Pathogenesis of *Candida albicans* [38]

Flavonoids have the ability to inhibit fungal growth through various key mechanisms, which involve causing disruptions in the plasma membrane, inducing mitochondrial dysfunction, and inhibiting important processes such as cell wall formation, cell division, ribonucleic acid and protein synthesis, as well as the activity of efflux pumps responsible for pumping out substances from the cells [41].

Role of Flavonoid in Disrupting Fungal Cell Membrane and Wall Formation

One of the ways in which flavonoids exert their anti-*Candida* effects is by disrupting the yeast cell membrane (**Figure** 5). *Candida* species have a cell wall composed of complex sugars and proteins, and flavonoids can interfere with the synthesis and assembly of these components, leading to the breakdown of the cell wall and eventual death of the yeast cells [1]. Flavonoids interfere with the fungal cell wall formation by inhibiting the synthesis of 1,3-β-D-glucan, leading to lysis of the fungal cells. Their derivative compounds then exhibit antifungal activity by damaging the cell membrane of the fungus [42]. Heung et al. discovered that myricetin, a derivates of flavones, exhibits antifungal effects on *Candida albicans* by impairing the integrity of the cell wall and significantly increasing membrane permeability. Moreover, *C. albicans* cells treated with myricetin demonstrated noticeable leakage of DNA and proteins, in comparison to the control group. Additionally, when *C. albicans* cells were treated with myricetin at a concentration equivalent to the minimum inhibitory concentration (MIC), there was a decrease in the binding of lipophilic probes, indicating that myricetin modifies the lipid components or arrangement within the cell membrane of *C. albicans*, resulting in enhanced membrane permeability [43].

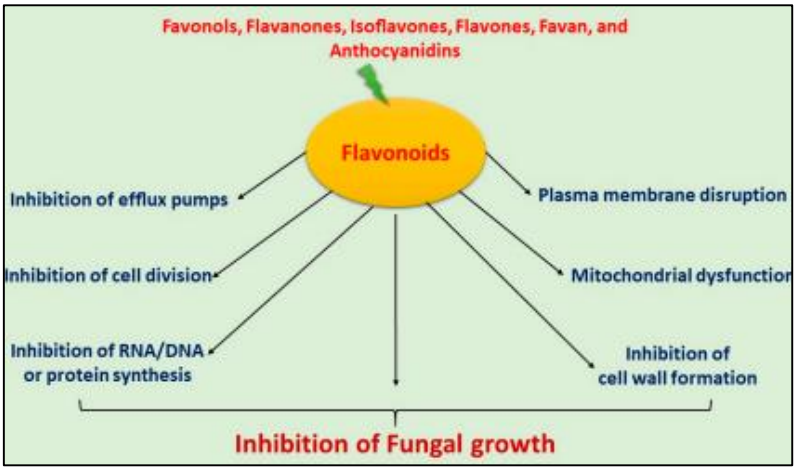

Figure 5. Antifungal activity of flavonoid [1]

Flavonoids can interact with the phospholipids that make up the cell membrane and alter their fluidity. This can affect the transport of ions and nutrients across the membrane, leading to membrane damage and cell death. Not only that, they also interfere with ergosterol synthesis, a key component of the fungal cell membrane, which is essential for fungal growth and survival. Flavonoids can inhibit the activity of enzymes involved in ergosterol biosynthesis, leading to a depletion of ergosterol in the cell membrane. This can cause membrane instability and damage, leading to cell death [1, 42-43]. This chemical structure also can generate reactive oxygen species (ROS) within the yeast cell. ROS can cause oxidative damage to membrane lipids, proteins, and DNA, leading to cell death [1, 5].

Biofilms, which are considered critical in the development of invasive fungal infections, contribute to phenotypic resistance along with genotypic resistance caused by resistant strains [44]. Intriguingly, a common feature shared by a limited number of antimycotics, such as miconazole (azoles), echinocandins, and liposomal formulations of amphotericin B (polyenes), is their ability to induce the generation of reactive oxygen species (ROS) within fungal (biofilm) cells [45]. It has been shown that Prenylflavanone 8PP has the potential to inhibit both azole-sensitive and resistant *C. albicans'* biofilms at 100µM through the collection and enhancement of endogenous ROS and reactive nitrogen intermediates [46]. The combination of baicalein and quercetin was found to down-regulate the expression of biofilm-specific genes, as revealed by real-time RT-PCR [47]. The efficacy of flavonoids in disrupting the biofilm of *Candida albicans* is noteworthy, especially considering that only a limited number of antimycotics demonstrate effectiveness against fungal biofilms [45].

Role of Flavonoid in Fungal Mitochondrial Dysfunction

Flavonoids have been shown to disrupt mitochondrial function in fungal cells, which can lead to a decrease in energy production and ultimately result in cell death. The mechanisms by which flavonoids induce mitochondrial dysfunction in fungi are not fully understood, but there are several proposed mechanisms: Inhibiting electron transport chain (ETC) complexes, and inducing mitochondrial membrane depolarization. Flavonoids can interfere with the activity of the ETC complexes, which are responsible for generating ATP through oxidative phosphorylation. This can lead to a decrease in ATP production, and accumulation of reactive oxygen species (ROS), causing oxidative damage to mitochondrial membranes, proteins, and DNA. Flavonoids can also disrupt the electrochemical gradient across the mitochondrial inner membrane, leading to mitochondrial depolarization. This can cause a decrease in ATP production and release of cytochrome c, which can activate apoptosis pathways and result in fungal cell death. A flavonoid derivative, Baicalein, has been shown to induce apoptosis in fungal cell through changes in mitochondrial membrane potential and increased intracellular ROS and upstream regulation of redox-related genes [1, 5, 15]. Under the influence of another derivates of flavonoid, Quercetin, the disruption of biofilms and the induction of mitochondrial dysfunction were observed. This mitochondrial dysfunction resulted in a decrease in mitochondrial redox levels and disruption of the mitochondrial antioxidant system. Additionally, there was an increase in intracellular reactive oxygen species (ROS) and a decrease in intracellular redox levels, indicating an overall disruption in antioxidant systems. As a consequence, DNA fragmentation occurred, which subsequently triggered apoptosis due to the DNA damage [48].

Role of Flavonoid in Inhibition of Fungal Cell Division

Flavonoids have been shown to inhibit fungal cell division by interfering with various cellular processes involved in mitosis, such as disrupting cell membrane integrity, inducing DNA damage, and disrupting microtubule dynamics. As discussed in previous point, flavonoids can interact with fungal cell membranes, which can lead to membrane damage and a decrease in membrane integrity. This can affect the function of membrane-bound proteins involved in cell cycle regulation, leading to cell cycle arrest and inhibition of fungal growth. They can also cause DNA damage by generating reactive oxygen species (ROS) or by intercalating into DNA strands, which can disrupt DNA replication and damage chromosomes, resulting in cell cycle arrest and inhibition of fungal growth. Lastly, flavonoids are thought to interact with microtubules, which are important for spindle formation and chromosome segregation during mitosis. Flavonoids can inhibit the polymerization of microtubules, which can result in aberrant spindle formation, chromosome misalignment, and ultimately, cell cycle arrest [1, 5, 15].

Flavonoid extracted from honey has shown inhibition effect to proliferation of *C. albicans* phenotype, causing lower infection rate, and disrupting fungal cell membrane integrity. The inhibition effect was measured using flow cytometry analysis and electron microscopy, showing the flavonoid extract

affects the hyphal transition by decreasing the G0/G1 phase and increasing the G2/M phase. Some other flavonoid derivatives have shown synergistic interaction with fluconazole, adding the suggested mechanism of cell cycle arrest in S phase in *C. albicans*. The crude extract containing flavonoids reduces efflux of the Cdr1 ABC transporter, which is the reason for fluconazole resistance. Daphnegiravone D, a prenylated flavonoid, has a cytotoxic effect and significantly inhibits cell division. Systematically, daphnegiravone D stops the G0/G1 phase and stimulates apoptosis by reducing the expression of cyclin E1, CDK2, and CDK4, as well as promoting caspase 3 and PARP cleavage [1, 5, 15].

The Future Research and Development Programs

Flavonoids and their antifungal bioactivity hold significant promise for uncovering novel therapeutic approaches. One challenging aspect of future research will involve further investigating the specific mechanisms by which flavonoids exert their antifungal effects, and understanding these mechanisms will provide valuable insights for the development of more potent and targeted antifungal agents.

Another area of focus will be the exploration of flavonoid-derived compounds, either through extraction from natural sources or synthesis, to enhance their antifungal activity. This includes structural modifications of flavonoids to optimize their pharmacological properties, such as improving their bioavailability, stability, and selectivity against fungal pathogens [49]. Novel delivery systems and formulations can also be explored to improve the efficacy and targeted delivery of flavonoid-based antifungal drugs. By investing in these areas of research and development, the next decade holds significant promise for advancing flavonoids as potent antifungal agents. The exploration of their therapeutic potential, coupled with rigorous scientific investigation, has the potential to revolutionize the field of antifungal therapy and provide new treatment options for invasive fungal infections. These compounds possess multifaceted properties, including antifungal and antioxidant activities, which make them highly attractive for combating invasive fungal infections [50]. The fact that flavonoids are naturally derived compounds further adds to their appeal, as they are often considered safe and exhibit a relatively low toxicity profile [51]. However, to fully harness the potential of flavonoids as antifungal drugs, further research is necessary. It is crucial to validate their safety and efficacy through rigorous preclinical and clinical trials. This will involve conducting comprehensive studies to determine optimal dosages, treatment regimens, and potential drug interactions.

CONCLUSION

Flavonoids have been extensively studied for their potential as anti-Candida agents. These natural compounds possess a wide range of biological activities, including anti-inflammatory, antioxidant, and antimicrobial properties. Flavonoids have been shown to inhibit the growth of *Candida* species, including the virulent *Candida albicans*, through various mechanisms, including disruption of cell membrane integrity, inhibition of mitochondrial function, and inhibition of fungal cell division. Flavonoids also exhibit anti-biofilm activity, which is of particular importance as *Candida* biofilms are highly resistant to conventional antifungal agents. These mechanisms of action can lead to decreased fungal growth and proliferation, and increased susceptibility of *Candida* cells to host immune responses and antifungal drugs. Studies have demonstrated that flavonoids such as quercetin, myricetin, kaempferol, and apigenin can effectively inhibit the growth of *Candida* species both in vitro and in vivo, with minimal toxicity to host cells. These flavonoids can also enhance the antifungal activity of conventional antifungal agents, indicating their potential as adjuvants in antifungal therapy.

The anti-*Candida* properties of flavonoids make them a promising area of research for the development of new antifungal therapies properties. Further studies are needed to elucidate their mechanisms of action and to determine their efficacy and safety in the clinical setting. Nevertheless, flavonoids represent a promising avenue for the development of novel antifungal agents to combat Candida infections.

REFERENCES

[1] M. S. Al Aboody and S. Mickymaray, "anti-fungal efficacy and mechanisms of flavonoids," *Antibiotics*, vol. 9, no. 2, 2020, doi: 10.3390/ANTIBIOTICS9020045.

[2] W. Nguyen, L. Grigori, E. Just, C. Santos, and D. Seleem, "The in vivo anti-Candida albicans activity of flavonoids," *J. Oral Biosci.*, vol. 63, no. 2, pp. 120–128, 2021, doi: 10.1016/J.JOB.2021.03.004.

[3] S. Safe, A. Jayaraman, R. S. Chapkin, M. Howard, K. Mohankumar, and R. Shrestha, "Flavonoids: structure–function and mechanisms of action and opportunities for drug development," *Toxicol. Res.*, vol. 37, no. 2, p. 147, Apr. 2021, doi: 10.1007/S43188-020-00080-Z.

[4] A. Ullah, S. Munir, S.L. Badshah, N. Khan, L. Ghani, B.G. Poulson, A.H. Emwas, and M. Jaremko, "Important Flavonoids and Their Role as a Therapeutic Agent," *Molecules*, vol. 25, no. 22, Nov. 2020, doi: 10.3390/MOLECULES25225243.

[5] A. Roy, A. Khan, I. Ahmad, S. Alghamdi, and B. S. Rajab, "Flavonoids a bioactive compound from medicinal plants and its therapeutic applications," *Biomed Res. Int.*, vol. 2022, 2022, doi: 10.1155/2022/5445291.

[6] S. Kumar and A. K. Pandey, "Chemistry and biological activities of flavonoids: an overview," *Sci. World J.*, vol. 2013, p. 16, 2013, doi: 10.1155/2013/162750.

[7] H. M. E. Willems, S. S. Ahmed, J. Liu, Z. Xu, and B. M. Peters, "Vulvovaginal candidiasis: A current understanding and burning questions," *J. Fungi*, vol. 6, no. 27, 2020, doi: doi: 10.3390/jof6010027.

[8] L. Jacob, M. John, M. Kalder, and K. Kostev, "Prevalence of vulvovaginal candidiasis in gynecological practices in Germany: A retrospective study of 954,186 patients," *Curr. Med. Mycol.*, vol. 4, no. 1, pp. 6–11, 2018, doi: 10.18502/cmm.4.1.27.

[9] Susilawati, K. Y. Rahadiyanto, M. Ramdja, Theodorus, and Hermansyah, "Uji diagnostik pemeriksaan multiplex-PCR pada penderita dengan klinis Kandidiasis vaginalis," *Biodiversitas*, vol. 20, no. 10, pp. 3063–3069, 2019.

[10] D. Rosati, M. Bruno, M. Jaeger, J. Ten Oever, and M. G. Netea, "Recurrent vulvovaginal candidiasis: An immunological perspective," *Microorganisms*, vol. 8, no. 2, pp. 1–14, 2020, doi: 10.3390/microorganisms8020144.

[11] D. W. Denning, M. Kneale, J. D. Sobel, and R. Rautemaa-Richardson, "Global burden of recurrent vulvovaginal candidiasis: a systematic review," *Lancet Infect. Dis.*, vol. 18, no. 11, pp. e339–e347, 2018, doi: 10.1016/S1473-3099(18)30103-8.

[12] S. Susilawati, C. Anwar, M. I. Saleh, and S. Salni, "Chemical Composition and Antifungal Activity of Morinda Citrifolia Fruit Extract," *Biosci. J.*, vol. 39, pp. 1–9, 2023.

[13] H. Hermansyah and S. Susilawati, "Gene expression changes and anti-proliferative effect of noni (morinda citrifolia) fruit extract analysed by real time-PCR," *Molekul*, vol. 12, no. 1, p. 37, 2017, doi: 10.20884/1.jm.2017.12.1.333.

[14] M. Ivanov, A. Kannan, D.S. Stojković, J. Glamočlija, R.C. Calhelha, I.C.F.R. Ferreira, D. Sanglard, and M. Soković, "Flavones, Flavonols, and Glycosylated Derivatives—Impact on Candida albicans Growth and Virulence, Expression of CDR1 and ERG11, Cytotoxicity," *Pharmaceuticals*, vol. 14, no. 1, pp. 1–12, Jan. 2021, doi: 10.3390/PH14010027.

[15] I. Gabriel, K. Rząd, E. Paluszkiewicz, and K. Kozłowska-Tylingo, "Antifungal activity of capridine β as a consequence of its biotransformation into metabolite affecting yeast topoisomerase II activity," *Pathogens*, vol. 10, no. 2, pp. 1–13, 2021, doi: 10.3390/PATHOGENS10020189.

[16] Q. Kanwal, I. Hussain, H. Latif Siddiqui, and A. Javaid, "Antifungal activity of flavonoids isolated from mango (Mangifera indica L.) leaves," *Nat. Prod. Res.*, vol. 24, no. 20, pp. 1907–1914, Dec. 2010, doi: 10.1080/14786419.2010.488628.

[17] L. Guo, Y. Li, X. Mao, R. Tao, B. Tao, and Z. Zhou, "Antifungal Activity of polymethoxylated flavonoids (PMFs)-loaded citral nanoemulsion against penicillium italicum by causing cell membrane damage," *J. Fungi*, vol. 8, no. 4, Apr. 2022, doi: 10.3390/JOF8040388.

[18] G. Simonetti, E. Brasili, and G. Pasqua, "Antifungal activity of phenolic and polyphenolic compounds from different matrices of vitis vinifera L. against human pathogens," *Molecules*, vol. 25, no. 16, Aug. 2020, doi: 10.3390/MOLECULES25163748.

[19] D. Seleem, V. Pardi, and R. M. Murata, "Review of flavonoids: A diverse group of natural compounds with anti-Candida albicans activity in vitro," *Arch. Oral* Biol., vol. 76, pp. 76–83, Apr. 2017, doi: 10.1016/J.ARCHORALBIO.2016.08.030.

[20] M. Ivanov, A. Kannan, D. Stojković, J. Glamočlija, S.G. Grdadolnik, D. Sanglard, and M. Soković, " *EXCLI J.*, vol. 19, p. 1436, 2020, doi: 10.17179/EXCLI2020-2987.

[21] A.H.W. Janabi, A.A. Kamboh, M. Saeed, L. Xiaoyu, J. BiBi, F. Majeed, M. Naveed, M.J. Mughal, N.A. Korejo, R. Kamboh, M. Alagawany, and H. Lv, Iran, "Flavonoid-rich foods (FRF): A promising nutraceutical approach against lifespan-shortening diseases," *Iran. J. Basic Med. Sci.*, vol. 23, no. 2, p. 140, 2020, doi: 10.22038/IJBMS.2019.35125.8353.

[22] Y.S. Ku, M.S. Ng, S.S. Cheng, A.W.Y. Lo, Z. Xiao, T.S. Shin, G. Chung, and H.M. Lam, "Understanding the Composition, Biosynthesis, Accumulation and Transport of Flavonoids in Crops for the Promotion of Crops as Healthy Sources of Flavonoids for Human Consumption,"

Nutrients, vol. 12, no. 6, pp. 1–23, Jun. 2020, doi: 10.3390/NU12061717.

[23] N. A. Evensen and P. C. Braun, "The effects of tea polyphenols on Candida albicans: Inhibition of biofilm formation and proteasome inactivation," *Can. J. Microbiol.*, vol. 55, no. 9, pp. 1033–1039, 2009, doi: 10.1139/W09-058.

[24] D. Seleem, B. Benso, J. Noguti, V. Pardi, and R. M. Murata, "In vitro and in vivo antifungal activity of lichochalcone-a against candida albicans biofilms," *PLoS One*, vol. 11, no. 6, Jun. 2016, doi: 10.1371/JOURNAL.PONE.0157188.

[25] M.Y. Lin, Z.L. Yuan, D.D. Hu, G.H. Hu, R.L. Zhang, H. Zhong, L. Yan, Y.Y. Jiang, J. Su, And Y. W. Chin, "Effect of loureirin A against Candida albicans biofilms," *Chin. J. Nat. Med.*, vol. 17, no. 8, pp. 616–623, Aug. 2019, doi: 10.1016/S1875-5364(19)30064-0.

[26] S.M. Nabavi, D. Šamec, M. Tomczyk, L. Milella, D. Russo, S. Habtemariam, I. Suntar, L. Rastrelli, M. Daglia, J. Xiao, F. Giampieri, M. Battino, E. Sobarzo-Sanchez, S.F. Nabavi, B. Yousefi, P. eandet, S. Xu, and S. Shirooie, "Flavonoid biosynthetic pathways in plants: Versatile targets for metabolic engineering," *Biotechnol. Adv.*, vol. 38, Jan. 2020, doi: 10.1016/J.BIOTECHADV.2018.11.005.

[27] W. Liu, Y. Feng, S. Yu, Z. Fan, X. Li, J. Li, and H. Yin, "The Flavonoid Biosynthesis Network in Plants," *Int. J. Mol. Sci.*, vol. 22, no. 23, Dec. 2021, doi: 10.3390/IJMS222312824.

[28] M. L. Falcone Ferreyra, S. P. Rius, and P. Casati, "Flavonoids: Biosynthesis, biological functions, and biotechnological applications," *Front. Plant Sci.*, vol. 3, no. SEP, p. 34352, Sep. 2012, doi: 10.3389/FPLS.2012.00222/BIBTEX.

[29] S. H. Han, B. G. Kim, J. A. Yoon, Y. Chong, and J. H. Ahn, "Synthesis of flavonoid o-pentosides by escherichia coli through engineering of nucleotide sugar pathways and glycosyltransferase," *Appl. Environ. Microbiol.*, vol. 80, no. 9, p. 2754, 2014, doi: 10.1128/AEM.03797-13.

[30] A. M. Pereira, H. Cidade, and M. E. Tiritan, "Stereoselective synthesis of flavonoids: a brief overview," *Mol. 2023, Vol. 28, Page 426*, vol. 28, no. 1, p. 426, Jan. 2023, doi: 10.3390/MOLECULES28010426.

[31] C. d'Enfert, A. K. Kaune, L. R. Alaban, S. Chakraborty, N. Cole, M. Delavy, D. Kosmala, and B. Marsaux, "The Impact of the Fungus-Host-Microbiota interplay upon Candida albicans infections: current knowledge and new perspectives," *FEMS Microbiol. Rev.*, vol. 45, no. 3, pp. 1–55, 2021.

[32] D. Kadosh and V. Mundodi, "A Re-Evaluation of the Relationship between Morphology and Pathogenicity in Candida Species," *J. Fungi 2020, Vol. 6, Page 13*, vol. 6, no. 1, p. 13, Jan. 2020, doi: 10.3390/JOF6010013.

[33] L. Mukaremera, K. K. Lee, H. M. Mora-Montes, and N. A. R. Gow, "Candida albicans yeast, pseudohyphal, and hyphal morphogenesis differentially affects immune recognition," *Front. Immunol.*, vol. 8, no. JUN, p. 266031, Jun. 2017, doi: 10.3389/FIMMU.2017.00629/BIBTEX.

[34] N. Sachivkina, I. Podoprigora, and D. Bokov, "Morphological characteristics of candida albicans, candida krusei, candida guilliermondii, and candida glabrata biofilms, and response to farnesol," *Vet. World*, vol. 14, no. 6, p. 1608, Jun. 2021, doi: 10.14202/VETWORLD.2021.1608-1614.

[35] J. Talapko, M. Juzbašić, T. Matijević, E. Pustijanac, S. Bekić, I. Kotris, and I. Škrlec, J. Fungi, "Candida albicans-the virulence factors and clinical manifestations of infection," *J. Fungi*, vol. 7, no. 2, pp. 1–19, 2021, doi: 10.3390/jof7020079.

[36] N. A. R. Gow, F. L. Van De Veerdonk, A. J. P. Brown, and M. G. Netea, "Candida albicans morphogenesis and host defence: Discriminating invasion from colonization," *Nat. Rev. Microbiol.*, vol. 10, no. 2, pp. 112–122, 2012, doi: 10.1038/nrmicro2711.

[37] J. P. Lopes and M. S. Lionakis, "Pathogenesis and virulence of Candida albicans," *Virulence*, vol. 13, no. 1. pp. 89–121, 2022. doi: 10.1080/21505594.2021.2019950.

[38] V. Sustr, P. Foessleitner, H. Kiss, and A. Farr, "Vulvovaginal candidosis: Current concepts, challenges and perspectives," *J. Fungi*, vol. 6, no. 4, pp. 1–14, 2020, doi: 10.3390/jof6040267.

[39] C. Susilawati, I. Anwar, Saleh, and Salni, *Respon Imun Tubuh terhadap Kandidiasis Vulvovaginal*. 2023.

[40] F. L. Mayer, D. Wilson, and B. Hube, "Candida albicans pathogenicity mechanisms," *Virulence*, vol. 4, no. 2, p. 119, Feb. 2013, doi: 10.4161/VIRU.22913.

[41] A. J. Yaqoub Hussain Hussein Hussein and A. Fauzi Al-Rubaye, "Antifungal efficacy of the crude flavonoid, terpenoid, and alkaloid extracted from myrtus communis l. against aspergillus species isolated from stored medicinal plants seeds in the iraqi markets," *J. Biotechnol. Res.*

Cent., vol. 15, no. 2, doi: 10.24126/jobrc.2021.15.2.613.

[42] S. Nuraeni, S. Rahmadanti, A. Fadilah, and Husnawati, "The spray of pegagan leaf extract as an antifungal of vulvovaginal candidiasis: a narrative review," Curr. *Biochem.*, vol. 7, no. 2, pp. 81–93, 2020, doi: 10.29244/cb.7.2.5.

[43] H. S. Lee and Y. Kim, "Myricetin disturbs the cell wall integrity and increases the membrane permeability of candida albicans," *J. Microbiol. Biotechnol.*, vol. 32, no. 1, pp. 37–45, 2022, doi: 10.4014/jmb.2110.10014.

[44] M. Gulati and C. J. Nobile, "Candida albicans biofilms: development, regulation, and molecular mechanisms," *Microbes Infect.*, vol. 18, no. 5, p. 310, May 2016, doi: 10.1016/J.MICINF.2016.01.002.

[45] N. Delattin, B. P. Cammue, and K. Thevissen, "Reactive oxygen species-inducing antifungal agents and their activity against fungal biofilms," *Future Med. Chem.*, vol. 6, no. 1, pp. 77–90, Jan. 2014, doi: 10.4155/FMC.13.189/FORMAT/EPUB.

[46] M. A. Peralta, M. G. Ortega, J. L. Cabrera, and M. G. Paraje, "The antioxidant activity of a prenyl flavonoid alters its antifungal toxicity on Candida albicans biofilms," *Food Chem. Toxicol.*, vol. 114, pp. 285–291, Apr. 2018, doi: 10.1016/J.FCT.2018.02.042.

[47] M. Janeczko, D. Gmur, E. Kochanowicz, K. Górka, and T. Skrzypek, "Inhibitory effect of a combination of baicalein and quercetin flavonoids against Candida albicans strains isolated from the female reproductive system," *Fungal Biol.*, vol. 126, no. 6–7, pp. 407–420, Jun. 2022, doi: 10.1016/J.FUNBIO.2022.05.002.

[48] M. S. Kwun and D. G. Lee, "Quercetin-induced yeast apoptosis through mitochondrial dysfunction under the accumulation of magnesium in Candida albicans," *Fungal Biol.*, vol. 124, no. 2, pp. 83–90, Feb. 2020, doi: 10.1016/J.FUNBIO.2019.11.009.

[49] S. H. Thilakarathna and H. P. Vasantha Rupasinghe, "Flavonoid bioavailability and attempts for bioavailability enhancement," *Nutrients*, vol. 5, no. 9, p. 3367, Aug. 2013, doi: 10.3390/NU5093367.

[50] A. N. Panche, A. D. Diwan, and S. R. Chandra, "Flavonoids: an overview," *J. Nutr. Sci.*, vol. 5, pp. 1–15, Jan. 2016, doi: 10.1017/JNS.2016.41.

[51] M. C. Dias, D. C. G. A. Pinto, and A. M. S. Silva, "Plant Flavonoids: Chemical Characteristics and Biological Activity," *Molecules*, vol. 26, no. 17, Sep. 2021, doi: 10.3390/MOLECULES26175377