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Review

Antimicrobial Activity of Dimeric Flavonoids

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Abstract: Distributed throughout the environment are various microorganisms such as bacteria, fungi, parasites, and viruses. Although many are part of the human microbiome, many are pathogenic and cause infections ranging from mild to severe. In recent years, the identification of multidrug-resistant microorganisms has become a serious public health problem. The resulting infections call into question the therapeutic capacity of health systems and lead to approximately 70,000 deaths annually worldwide. The progressive resistance to antibiotics and antifungals has been a major challenge for the medical and pharmaceutical community, requiring the search for new compounds with antimicrobial properties. Several studies have demonstrated the potential of natural and synthesized flavonoids, especially the dimers of these molecules. In this review are presented many examples of dimeric flavonoids that have demonstrated antimicrobial activity against viruses, like influenza and Human Immunodeficiency Virus (HIV), protozoal infections, such as Leishmaniasis and Malaria, fungal infections by *Candida albicans* and *Cryptococcus neoformans*, and bacterial infections caused, for example, by *Staphylococcus aureus* and *Escherichia coli*. In the pursuit to find potential safe agents for therapy in microbial infections, natural dimeric flavonoids are an option not only for the antimicrobial activity, but also for the low toxicity usually associated with these compounds when compared to classic antimicrobials.

Keywords: flavonoids; dimeric flavonoids; antimicrobial activity; antiviral; antifungal; antibacterial; antiparasitic

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1. Introduction

During the last few decades, the incidence of microbial infections has increased significantly, which culminates in high morbidity and mortality rates [1]. This is due to the existence of more immunocompromised individuals (due to HIV infection, chemotherapy, and radiotherapy treatments), use of immunosuppressants, an increased number of hospitalized patients, invasive devices and procedures in medical practice and the evolution of virulence and resistance mechanisms to antimicrobial agents [2–4].

Although antibiotics and antifungals have a wide spectrum and different mechanisms of action, their incorrect and indiscriminate use has had the consequence of the increase in resistance mechanisms developed by microorganisms [5]. The medical community has limited options for resolving bacterial and fungal infections [6,7].

Parasitic diseases affect over 1 billion people all over the world, specifically, parasitic diseases such as malaria and schistosomiasis, leading to approximately 1 million deaths throughout the world [8]. Drug resistance associated with the treatment of these infections

are widespread and, even with some genes already found related to resistance for available therapy, the mechanisms are not completely understood [9].

Antiviral resistance has been widely studied and is commonly related with factors that involve a decrease in host immunity and prolongs the duration of treatment [8]. Consequently, there is an increase in side effects due to the toxicity caused using second-line antivirals and, in cases of serious illness, death due to progressive viral infection when there is no cure available [10].

The efficacy of a therapeutic agent may be affected by the development of withdrawal mechanisms from the first time it is applied [11]. The increasing resistance to antifungals and antibiotics available for clinical practice has been a major challenge for the medical and pharmaceutical communities, requiring the search for new compounds with antimicrobial properties (e.g., of plant origin). Besides that, this strategy may involve the search for new compounds to counteract resistance mechanisms or make it possible to reduce the dose of the antimicrobial and, consequently, its toxicity and adverse effects [11,12].

Several studies have proven the individual or synergistic antimicrobial potential of natural and synthetic flavonoids, against drug-resistant fungi [13], bacteria [14], virus [15] and parasites [16].

Flavonoids are a versatile group of phenolic compounds produced as secondary metabolites by plants and, therefore, existing in the human diet (e.g., present in fruit, vegetables, cereals, wine, and various teas) [17]. These compounds are responsible for the coloration of leaves, flowers and fruits, and have a fundamental role in the protection of plants as oxidizing and microbial agents [18]. As a class of polyphenols, flavonoids can be divided in flavones (apigenin), flavanones (naringenin), flavans (catechin), flavonoid glycosides, flavonols (quercetin), flavonolignan (silibinin), chalcones (butein), isoflavones (genistein), aurones (aureusidin), leucoanthocynidins (leucopelargonidin) and neoflavonoids (neoflavones) [19–21].

Flavonoids were described in the literature as processing anti-allergic [22–25], anti-inflammatory [25–30], immunomodulatory [31–33], antitumor [33–36] and antimicrobial [15,21,37–41] properties, which are the reasons explaining their great interest in the food, pharmaceutical and medical industries. Their toxicity levels are greatly reduced and are therefore currently critical for the development of new medicines [11,13,21,42–44].

Dimeric flavonoids are a class of flavonoids that consists of the same or diverse flavonoid units connected by C-C bonds or by C-O-C bonds. These dimers are joined in a symmetrical or unsymmetrical manner through an alkyl or an alkoxy-based linker of varying length [45,46]. Mostly, dimeric compounds are formed by dimers of flavone–flavone, flavone–flavonone, and flavonone–flavonone subunits, as well as dimers of chalcones and isoflavones [45]. These compounds are called bis-flavonoids when they have two equal units or biflavonoids when there are two different units in the dimer structure. Since several dimeric compounds have been identified, the scientific community have been interested in their antimicrobial properties [45,46].

The main goal of this review is summarizing the remarks of several published studies on the use of dimeric flavonoids as antimicrobial agents, analyzing their role in aiding or resolving fungal, bacterial, parasitic, and viral infections. Thus, this review aims to outline the potential mechanism of actions of dimeric flavonoids studied in vitro and in vivo, and the perspectives of their use as multi-targets agents or conjugated with antimicrobials already known and applied in the treatment of infections.

A thorough search of the relevant scientific databases, including Web of Science, ScienceDirect, Scopus, PubMed, and Google Scholar, was conducted. The keyword combinations used in all databases were as follows: (antimicrobial resistance AND antibacterial resistance) OR (antimicrobial resistance AND antifungal resistance) OR (antimicrobial resistance AND antiparasitic resistance); (flavonoids AND biological properties) OR (flavonoids and antimicrobial activity) OR (flavonoids AND (dimeric flavonoids OR dimeric compounds); (dimeric flavonoids AND antimicrobial activity) OR (dimeric flavonoids AND antiviral activity) OR (dimeric flavonoids AND antifungal activity) OR (dimeric flavonoids

AND (antiprotozoal OR anthelmintic activity) OR (dimeric flavonoids AND antibacterial activity); (dimeric flavonoids AND (clinical studies OR in vivo studies OR pre-clinic tests OR assays in animal models).

Preliminary reading and analysis allowed the selection of several studies published between 2000 and 2023, which were later thoroughly analyzed. Studies written in Portuguese, Brazilian and English were selected.

Abstracts of selected titles were reviewed based on some inclusion and exclusion criteria. The articles that described the antimicrobial assays but did not state the respective control experiments, as well as studies describing it in a contradictory or unclear manner, were excluded from the review.

2. Dimeric Flavonoids

In 1929, ginkgetin, the first dimeric flavonoid, was separated from Ginkgo biloba by Furukawa and opened a new path for the discovery of more than 500,000 of those compounds, such as amenthoflavone, agatisflavone, cupressoflavone, hynoquiflavone and robustaflavone [47]. Due to their chemical and biological properties, there has been a great evolution regarding phytochemical chemical studies for the manipulation, molecular rearrangement strategies, identification, and synthesis of new bioactive dimeric flavonoids with potentiated characteristics [46].

Dimeric flavonoids are extensively studied for their pharmacological properties, as they have low toxicity in human cells [46,48–51], which has opened new routes to find and synthetize new drugs against pathogens.

Recently, research has extensively reported that the biologic activity of these compounds is higher than monomeric flavonoids, due to the high number of hydroxyl groups that reduce hydrophobicity [46,52–56].

Despite being very promising compounds, there are very few that are completely studied, with elucidated mechanisms of action and with their toxicity investigated [46].

2.1. Antiviral Activity of Dimeric Flavonoids

Dimeric flavonoids can act against many RNA and DNA viruses by blocking different stages of virus life cycle: fixation and entry in the cells, interference with replication and formation, maturation, and liberation of new mature viral particles [46]. In addition, these compounds might be indirect inhibitors by interacting with immune cells of the host [46].

Due to resistance to antiviral drugs, it has become vital to search for more compounds that can reduce the side effects, viral latency, and recurrence of infections. Nonetheless, the emergence of new viruses brings many obstacles to medicine [46]. These dimeric compounds seem to be more promising than flavonoids due to their greater physical–chemical stability during tests of pharmacokinetic parameters [57].

Dimeric flavonoids, such as amentoflavone, have become compounds of interest due to their important antiviral effects, mainly as protease inhibitors [58]. Several reports describe the activity of natural products against coronaviruses (CoV), with the main target being viral replication. Of these, numerous flavonoids, such as quercetin, generated strong antiviral activity, affecting SARS-CoV, MERS-CoV, and SARS-CoV-2 proteases [57]. In addition to the characteristic symptoms of COVID-19, SARS-CoV-2 infection can also result in complications, one of the most worrying being cytokine storm that can lead, in the worst-case scenario, to multiple organ failure and death [59,60].

Bearing this in mind, it is also necessary to use drugs to treat these diseases which, in addition to having a direct antiviral effect, also can modulate the immune response triggered by the infection. Because of their diverse biological activities, dimeric flavonoids may be used in combination with antivirals currently used in the clinic [46]. Table 1 lists some of the compounds that are referred in the literature as possessing effective antiviral activity.

Table 1. Antiviral activity in vitro of dimeric flavonoids.

Virus	Compound	Conclusions	Literature Reference
SARS-CoV-2	Agathisflavone	Replication block by M^{pro} protease inhibition. Reduces TNF-α (tumor necrosis factor-alpha) levels in infected cells.	[57]
	5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one	Interruption of viral RNA replication by blocking the Chymotrypsinlike protease	[61]
Influenza	Ginkgetin	Inhibits sialidase activity.	[62]
	Hinokiflavone		[63]
	Agathisflavone	Inhibits neuraminidase activity.	[64]
Human immunodeficiency virus (HIV)	Robustaflavone and hinokiflavone	Blocks reverse transcriptase activity.	[65]
	Morelloflavone	Activity against HIV-1.	
Epstein−Barr virus (EBV)	Garcinianin and talbotaflavone	Inhibit the 12-O-tetradecanoylphorbol-13-acetate-(TPA)-induced Epstein−Barr virus early antigen (EBV-EA) activation in Raji cells.	[66]
	3′, 4′, 5, 7-tetrahydroxyflavone	Inhibit the reactivation of EBV through early genes (Zta and Rta) block and interfering with binding of transcription factor Sp1.	[67]
Dengue virus	Hinokiflavone and Amentoflavone	Inhibits RNA-dependant RNA polymerase (DV-NS5 RdRp).	[48,68]
	Sotetsuflavone and robustaflavone	Inhibits dengue virus NS5 RNA dependent RNA polymerase.	[68]
	Agathisflavone	Inhibits NS2B-NS3 protease.	[69]
	Podocarpusflavone A	Inhibits the DV-NS5.	[48]
Hepatitis B virus (HBV)	Robustaflavone	Inhibits the DNA polymerase.	[70]
	Sikokianin A	Reduces HBsAg secretion.	[71]
Hepatitis C virus (HBC)	Amentoflavone	Deregulates all the virus life cycle, including viral entry, replication, and translation. Inhibitor of NS5A.	[72]
Herpes simplex virus (HSV)	Amentoflavone	Affects the expression of UL52 (early gene), UL54 (immediate-early gene) and UL27 (late gene). More active against HSV-1.	[73]
	Agathisflavone	Active against HSV-1 and HSV-2.	[74]
	Strychnobiflavone	Interferes with the initial stages of viral infection and reduces HSV-1 protein expression.	[75]
	Ginkgetin	Activity against HSV-1 and HSV-2. Inhibits the transcription step in the protein synthesis of HSV-infected cells.	[76]
Coxsackievirus B3	Amentoflavone	Inhibits fatty acid synthase.	[77]

Abbreviations: TNF-α, tumor necrosis factor-α; RNA, ribonucleic acid; HIV, Human immunodeficiency virus; DNA, deoxyribonucleic acidHBV, Hepatitis B virus; HBC, Hepatitis C virus; HSV, Herpes simplex virus.

Chaves, O. et al. used not only the dimeric flavonoid agatisflavone, but also its natural monomer apigenin, and demonstrated that the dimeric form increased the antiviral capacity of flavonoids, which might be explained by the top number of hydrophobic contacts by the number of aromatic rings [57]. The study of Y. Lin et al. also showed that the presence of a greater number of hydroxylated groups and at least one flavone unit in dimeric flavonoid compounds are essential for their antiviral activity. On the other hand, the compounds studied can become inactive when the hydroxyl groups are methylated [65].

2.2. Antifungal Activity of Dimeric Flavonoids

The antifungal activity and, respectively, mechanisms of action of dimeric flavonoids were investigated against several pathogenic fungal strains, such as *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Penicillium marneffei*, *Alternaria alternata*, *Fusarium culmorum*, and *Cladosporium oxysporum* [46]. The diagram of Figure 1 shows the main targets of dimeric flavonoids against fungi described in the literature.

Dimeric flavonoids can form complexes with soluble proteins in fungal cell walls and the lipophilic nature of these compounds makes them capable of disrupting fungal membranes [46,78,79].

Regarding these two types of fungus, there have been dimeric flavonoids, like isoginkgetin, that have demonstrated antifungal activity against *Cryptococcus neoformans* and *Aspergillous fumigatus*, a yeast, and a filamentous fungus, respectively. These data show that these types of compounds have a large spectrum of action [80].

According to the literature, one of the main characteristics of these compounds is their ability to inhibit the growth and multiplication of fungus, like *Candida albicans* and *Alternaria alternata*, and the growth of spores [81,82].

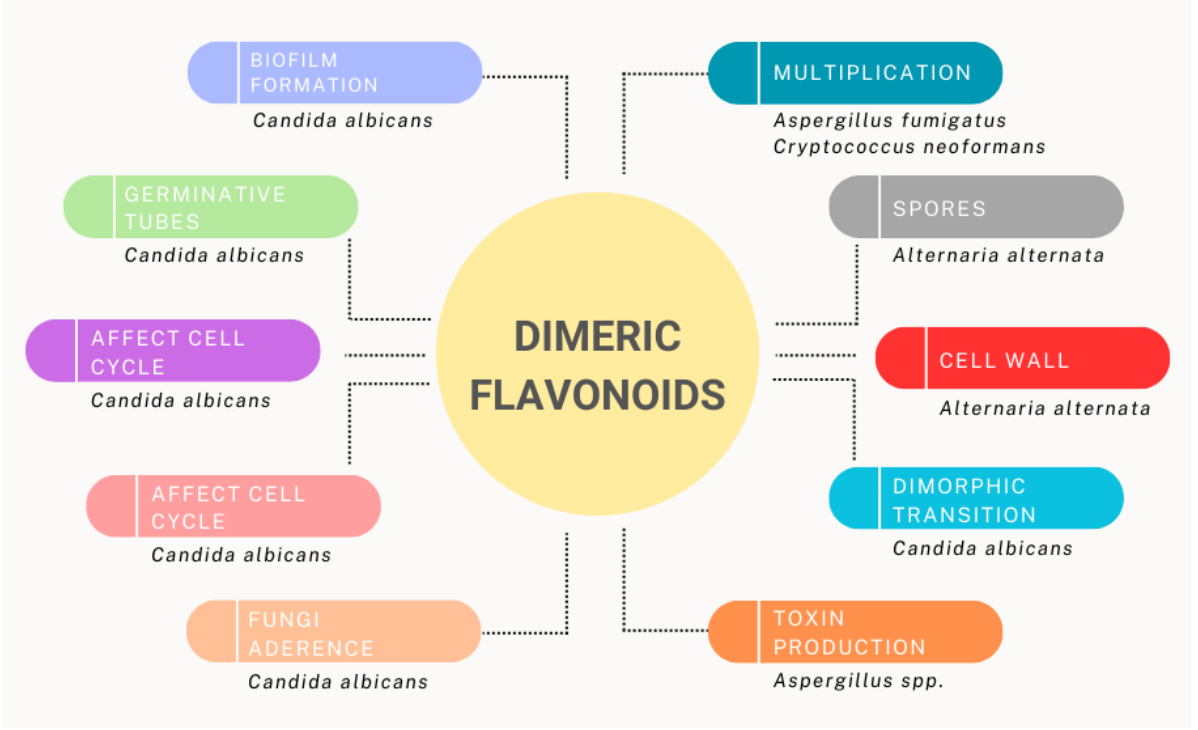

Figure 1. Main targets of dimeric flavonoids against fungi [46].

When evaluating a possible interference of dimeric flavonoid compounds with virulence factors that determine the pathogenicity of fungi, studies have shown that amentoflavone enables *Candida albicans* to make a dimorphic transition due to a stress response by the accumulation of trehalose and bilobetin, which is able to inhibit the growth of germinating tubes from *Cladosporium oxysporum* and *Fusarium culmorum* [82,83]. As far as the production of toxins is concerned, the compounds amentoflavone, 7,7″-Dimethoxyagastisflavone, 6,6″-bigenkwanin, and tetramethoxy-6,6″-bigenkwanin, isolated from the *Ouratea* species, inhibited the production of aflatoxins B1 and B2 from *Aspergillus flavus*, and the maximum effect happened at 10 µg/mL [84].

In the case of *Alternaria alternata*, ginkgetin and 7-O-methylamentoflavone provoked cell wall changes by an hydrophobic interaction [82].

Since biofilms are an enormous obstacle against antifungal agents, Freitas et al. tested if proanthocyadnidin polymeric tannins from the *Stryphnodendron adstringens* stem bark with antifungal activity against *Candida albicans* were also active during biofilm formation and on pre-formed biofilms for *Candida* spp. The best results for *Candida* spp. were for *C. albicans*, with MICs of 3.91 and 0,48 mg/L, that represented the inhibition of planktonic and dispersion cells, respectively [85]. In conclusion, their study highlighted the potential of those dimeric compounds to inhibit the formation of those communities of yeasts [85].

Additionally, some synthetic antifungal dimeric flavonoids were generally more active against *Aspergillus niger* (MICs of 0.2, 0.0013 and 0.4 µmol/mL of dimers) when compared to correspondent monomeric compounds of apigenin [55].

Considering all these findings, dimeric flavonoids that possess inherent antifungal activity (Table 2) could be a strategy for future antifungal therapy [86].

Interestingly, dimeric flavonoids, like amentoflavone and other compounds consisting of flavanone–flavone units (like 2,3-dihydrosciadopitysin) with a methoxyl group absent, were inactive or weakly effective [58].

Table 2. Dimeric flavonoid antifungal activity.

Fungus	Compound	Conclusions	Literature Reference
Candida albicans	Amentoflavone	Fungistatic. Affects cell cycle progress during S-phase. Interrupts dimorphic transition.	[81]
		Enhances the intracellular trehalose level, which induces a stress response in fungal cells.	[87]
	Quercetin	Inhibits fungal adherence and biofilm formation.	[83]
	Kaempferol-3,40-dimethylether	Activates macrophages and increases lysosomal activity.	[88]
	Kaempferol, canthin-6-one, and morin)	Cell membrane damage.	[89]
	Proanthocyanidin	Inhibits proliferation and dispersion cells from pre-formed biofilms.	[85]
Aspergillus flavus	Amentoflavone, 7,7″-Dimethoxyagastisflavone, 6,6″-bigenkwanin, and tetramethoxy-6,6″-bigenkwanin	Reduces the production of aflatoxin B1 (AFB1) and B2 (AFB2).	[84]
Aspergillus fumigatus	Isoginkgetin	Growth inhibition.	[80]
Cryptococcus neoformas	Isoginkgetin Podocarpusflavone	Growth inhibition.	[80]
Fusarium culmorum	Bilobetin	Inhibits the growth of germinating tubes.	[82]
Cladosporium oxysporum	Bilobetin	Inhibits the growth of germinating tubes.	[82]
Alternaria alternata	Ginkgetin and 7-O-methylamentoflavone	Inhibits the growth of fungal spores. Small changes in the cell wall.	[82]

Abbreviations: AFB1, Aflatoxin B1; AFB2, Aflatoxin B2.

2.3. Antiparasitic Activity of Dimeric Flavonoids

Parasitic infections are responsible for a great strain on health systems and affect millions of people around the world [90].

According to the literature, some dimeric flavonoids, such as morelloflavone and strychnobiflavone, show activity against both promastigote and amastigote forms [91]. Considering virulence factors of *Leishmania* spp., studies showed that the dimeric flavonoids lanaroflavone, podocarpusflavone A, amentoflavone, and podocarpusflavone B, inhibited the action of a zinc-dependent metalloprotease, existing in amastigote and proamastigote forms of *L. major* and *L. panamensis*, which reduces the ability of parasites to adhere to macrophages by interaction with fibronectin [46,92].

As for malaria disease, although there are medications such as chloroquine, vector control, and vaccines (about 40% effective) capable of controlling transmission, it remains a serious parasitic infection [46]. Dimeric flavonoids, such as lanaroflavone, methylenebis-santin and 3″,4′,4‴,5,5″,7,7″-heptahydroxy-3,8-biflavanone, demonstrated high activity against *Plasmodium falciparum*, in some cases by inhibiting important enzymes [51,93,94]. Weniger et al. and Kunert et al. stated that the patter of methylation of the compounds are determinants for antiplasmodial activity [94,95].

Other dimeric compounds, such as 2″,3″-Dihydroochnaflavone and brachydins B and C, showed important antiparasitic activity against *Trypanosoma cruzi* amastigotes and trypomastigotes forms, and inhibited its capacity to invade [96,97].

During this review, no dimeric compounds were found with antiparasitic activity against helminths.

Table 3 summarize some dimeric flavonoids that demonstrated ability to act against protozoa.

Table 3. Activity of dimeric flavonoids against protozoa.

Protozoa	Compound	Conclusions	Literature Reference
Plasmodium falciparum	3″,4′,4‴,5,5″,7,7″-heptahydroxy-3,8-biflavanone	Inhibition of α-glucosidase and aromatase.	[51]
	Lanaroflavone	Mechanism of action unknown.	[94]
	7,4′,7″-tri-O-methylamentoflavone	Mechanism of action unknown.	[95]
	Methylenebissantin	Inhibits enoyl-ACP reductase.	[93]
	3,3″-di(7,4″-dihydroxyflavanone-3-yl)	Mechanism of action unknown.	[98,99]
Leishmania panamensis	Lanaroflavone Podocarpusflavone A Podocarpusflavone B Amentoflavona	Interact with Glycoprotein 63.	[92]
Leishmania infantum	Strychnobiflavone	Causes depolarization of parasitic mitochondria.	[100]
	Amentoflavone	Activity against intracellular amastigotes.	[95]
Leishmania donovani	2,3-Dihydrohinokiflavone	Tested on axenic amastigotes.	[91]
Leishmania mexicana	Morelloflavone and Acetate	Interact with recombinant cysteine protease type 2.8	[91]
Leishmania amazonensis	Amentoflavone and robustaflavone	Effective antioxidant activity by increasing nitric oxide (NO) production in macrophages. Strong activity against promastigote and amastigote forms.	[91]
	7-O-methyl ochnaflavone	Activity against promastigote forms.	[101]
	Brachydin	Reduces the number of amastigotes and infected macrophages. Presents a synergic effect with amphotericin B. Also showed ability to induce damage in Golgi apparatus by accumulation of vesicles.	[102]
Trypanosoma cruzi	2″,3″-Dihydroochnaflavone	Kills approximately 62% of amastigote forms and 100% of trypomastigotes in infected murine macrophages. The mechanism is unknown. It is also able to inhibit topoisomerase I and topoisomerase II-α, which may be the cause of mitochondrial alterations in the parasitic form.	[96]
	Brachydin B and C	Inhibits the parasite invasion and its intracellular multiplication in host cells, reducing parasitemia.	[97]

Abbreviations: NO, nitric oxide.

The fact that dimeric flavonoids tested with commonly used anti-parasitic drugs revealed the absence of competition/interaction may represent an important strategy that allows reducing the dose, adverse effects, time, and cost of treatments, overcoming the weak activity of some medications when administered individually [103].

Additionally, Ichino et al. and Boniface and Ferreira used the liquiritigenin dimer 3,3″-di(7,4″-dihydroxyflavanone-3-yl) and the monomeric liquiritigenin and stated that the monomeric form did not have antiplasmodial activity [98,99]. Also, Thévenin et al. found that the synthetic compounds of methylenebis(chalcone)s were more active against parasites [104]. These extra data comparing dimeric and monomeric forms enhance the potential of dimeric flavonoid investigation for antiparasitic effects [104].

2.4. Antibacterial Activity of Dimeric Flavonoids

Antibacterial resistance has become a problem of public health recognized all around the globe [5]. To find alternatives for resolving infections caused by multi-drug-resistant bacteria, the medical and pharmacological industry need to search for new products that have functions like those of available antibiotics [41].

Although the mechanism of action of antibacterial dimeric flavonoids might not be elucidated, some authors may assume that they may act in a similar way to monomeric compounds, as show in Figure 2 [46].

In general, these compounds are more potent in Gram-positive rather than Gram-negative bacteria, due to the differences between the cell wall of those two groups of bacteria, especially due to the repulsive effect of lipopolysaccharides present in Gram-negative bacteria [80]. One of the mechanisms characterized is the disruption of plasma membranes [46,80,105,106]. For example, isoginkgetin and podocarpusflavone MICs for *S. aureus* and *E. faecalis* were 60.0 μg/mL, which showed a moderate activity; for Gram-negative *E. coli* and *P. aeruginosa*, isoginkgetin MICs were 130 μg/mL and podocarpusflavone were 250 and 60 μg/mL, respectively, which represented a lower activity [86].

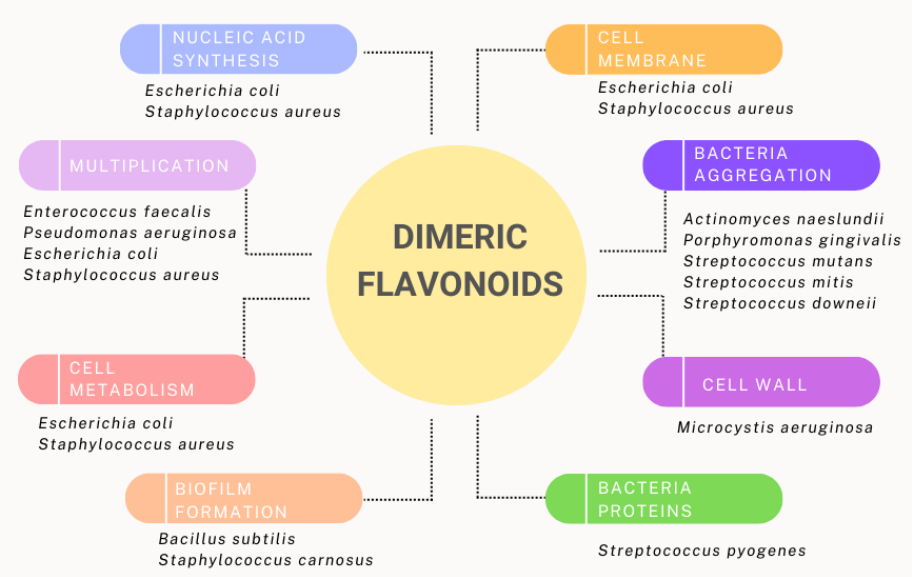

Figure 2. Main targets of dimeric flavonoids against bacteria [46].

Other compounds, like macrophylloflavone, can interfere with nucleic acid synthesis and other dimeric flavonoids [105]. Additionally, and just like the compound 3″,4′,4‴,5,5″,7,7″-heptahydoxy-3-8″-biflavone, they can also interfere with the metabolism of the bacterial cell and uptake of crucial nutrients, like glucose [105,107].

As already shown for fungus, dimeric flavonoids like agatisflavone, amentoflavone, tetrahydroamentoflavone (THAF) and fukugiside can inhibit the bacterial growth and inhibit the biofilm formation, such as *Bacillus subtilis*, *Staphylococcus carnosus* and *Streptococcus pyogenes* [108,109].

The investigation of Linden et al. investigation a remarkable antibacterial activity of THAF against Gram-positive microorganisms: *B. subtilis*, with an MIC and MBC of 0.063 mg/mL and a bactericidal effect of 0.125 mg/mL for *S. carnosus*. In this case, the results stated for the first time that dimerization and a reduced C–ring in dimeric flavonoids, such as in THAF, may be the answers to justify the highest antibacterial activity. Regarding biofilm inhibition, THAF was able to inhibit the biofilm formation of methicillin-resistant *S. aureus* (MRSA) [108].

The findings of Nandu et al. on fukugiside showed that a concentration of 80 µg/mL reduces an *S. pyogenes* biofilm by 91% by minimizing the cell surface hydrophobicity, which do not rely on bacterial viability [109]. Furthermore, this dimeric flavonoid was also able to interfere with an important virulence factor—M proteins—that have antiphagocytic functions, enhancing *S. pyogenes* rate survival in human tissues and fluids. These proteins are encoded by the *emm* gene, which is positively regulated by *mga*. Fukugiside downregulated *mga*, which represented the possible prevention of systemic spread [109].

In short, Table 4 summarizes dimeric flavonoids that have antibacterial activity and the mechanism of action, when elucidate.

The study of Lee et al. showed that the dose-dependent killing of *M. aeruginosa KW* could be due to another variety of flavonoids in the *S. tamariscina* extract. For example, in that work, apigenin, a monomer of amentoflavone, also had cyanobacterial-killing effects. However, those effects were insufficient compared to the ones obtained for amentoflavone [106].

Table 4. Activity of dimeric flavonoids in bacteria.

Bacteria	Compound	Conclusions	Literature Reference
Staphylococcus aureus	7, 4′, 7″, 4‴-Tetramethoxy amenthoflavone	The lipophilic nature of the molecules and the external porous peptide cell wall structure of Gram-positive bacteria determined their effect. In Gram-negative bacteria, growth inhibition is lower.	[80]
	Macrophylloflavone 18	Inhibits nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, and porins in cell membranes.	[105]
	Isoginkgetin	Growth inhibition.	[80]
	Podocarpusflavone—A	Mechanism of action unknown.	[80]
	Manniflavanone	Mechanism of action unknown.	[110]
Escherichia coli	Macrophylloflavone 18	Inhibits nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, and porins in cell membranes.	[105]
	Isoginkgetin	Growth inhibition.	[80]
	Ericoside	Mechanism of action unknown.	[111]
Bacillus subtilis and *Staphylococcus carnosus*	Agatisflavone 2, amentoflavone 1, and Tetrahydroamentoflavone (THAF)	Inhibition of biofilm formation. Dimerization and a reduced C ring contribute to greater activity of the compounds.	[108]
Streptococcus pyogenes	Fukugiside	Exhibited concentration-dependent biofilm inhibition by destabilizing the biofilm matrix and by inhibiting M proteins.	[109]
Pseudomonas aeruginosa	Ochnaflavone and ochnaflavone 7-O-methylether 15c	Mechanism of action unknown.	[112]
Microcystis aeruginosa	Amentoflavone	Bacteria lose their round shape and eventually succumb completely. Affects the peptidoglycan layer and reduces pressure, which ends with the leaking of cell contents. Effects are dose-dependent.	[106]
Enterococcus faecalis	Podocarpusflavone—A	Mechanism of action unknown.	[80]
	Manniflavanone	Mechanism of action unknown.	[88]
	Isoginkgetin	Growth inhibition.	[80]
	Ochnaflavone and ochnaflavone 7-O-methylether 15c	Mechanism of action unknown.	[112]
Actinomyces naeslundii, Porphyromonas gingivalis, Streptococcus mutans, Streptococcus mitis and *Streptococcus downeii*	3″,4′,4‴,5,5″,7,7″-heptahydoxy-3-8″-biflavone	Inhibition of glucan synthesis, glucose uptake and metabolism. Induces bacterial aggregation.	[107]
Klebsiella pneumoniae	Ericoside	Mechanism of action unknown.	[111]

Abbreviations: THAF, Tetrahydroamentoflavone.

Interestingly, Bitchagno et al. found that the antibacterial activity of the dimeric flavonoid ericoside was higher for drug-resistant *E. coli* AG100 (MIC = 64 μg/mL) and for *Klebsiella pneumoniae* ATCC11296 (128 μg/mL) than for monomeric taxifolin 3-O-rhamnopyranoside, in which the MICs found were >128 μg/mL [111].

2.5. Potential of Dimeric Flavonoids as Antimicrobials: Form Lab to Clinics

In the literature, it is easy to find studies that have tested many flavonoids compounds in vivo, using animal models, to assess their antimicrobial activity and toxicity levels, and the results are starting to be potentiated in clinical trials [41].

Recent studies demonstrate that topically applied flavonoids, specially flavonols and flavanols are effective when used via oral and vaginal mucosa routes [44]. The work of Araújo et. al, showed that the in vivo tests in mice with a vaginal cream with an extract from *Syngonanthus nitens* scapes (having flavonoids as the bioactive compounds) eliminated vaginal Candida albicans, with only signs of inflammatory infiltrate and ulcerations that indicated a previous infectious process in the local mucosa [113]. Simonetti et al. also tested in vivo grape seed extract polymeric flavan-3-ols that inhibited C. albicans load in vaginal candidiasis in mice [114]. Furthermore, research from Seleem et al. showed that the compound lichochalcone-A, applied topically in the oral cavities of immunosuppressed

mice, not only resulted in an extensively reduced fungal load, but also did not have significantly toxicological effects, with the absence of tissue necrosis [115].

Regarding in vivo activity against parasites, according to Marín et al., none of the nine flavonoids tested in mice infected with *T. cruzi* had significant toxicity and the parasitic charge was extensively lower when compared with a benznidazole control [116]. Additionally, those compounds changed the levels of the anti-*T. cruzi* antibody during the chronic stage [116]. An in vivo study with 2′-Hydroxyflavanone showed that this flavonoid reduced the lesion size and *L. amazonensis* load in a murine model of cutaneous leishmaniasis [117]. Pereira et al. stated the in vivo schistosomicidal activities of oral treatment with chalcones against Schistosoma mansoni worms, and the results showed that in mice there occurred a total worm reduction [118].

The effects on acute lung injury induced by the influenza A virus in mice of an extract from *Scutellaria baicalensis* root with bioactive flavonoids showed that oral administration protected the infected animals by decreasing the lung virus load by affecting the production of reduced haemagglutinin and inhibiting neuraminidase activity [119]. Based on the Ma et al. study, the authors highlighted the in vivo activity of oxazinyl flavonoids against tobacco mosaic virus [120].

The clinical test conducted with *Plantago lanceolata* extracts, with the in vitro antimicrobial activity of flavonoids, demonstrated that the individuals that had a *P. lanceolata* mouth rinse presented a significant decrease in streptococci compared to the placebo group [121]. Even not significantly, the study stated a minor decrease for lactobacilli counts after the treatment [121]. Another in vivo study with mice showed that an ethanolic extract had a powerful antibacterial action against *S. aureus*, *P. aeruginosa* and *Listeria monocytogenes*, attributed to the high content of catechin, epicatechin gallate and epicatechin, and may be useful as an antiseptic solution [122].

Given these data, it is assumed that the study of flavonoids has increased. However, there are few studies with animal models and clinical trials regarding the antimicrobial activity of dimeric flavonoid compounds [46].

Rocha et al. performed a study in mice that showed that brachydin B, a dimeric compound from *Arrabidea Brachypoda*, reduced the parasitemia in the infected animals with *L. amazonensis* [102]. The oral administration suggested that the compound is absorbed by the oral route and can reduce parasitemia [102]. The same compound was also tested against *T. cruzi* in mice and was possible to highlight the low toxicity and decrease in parasitemia and mortality [97,102]. Therefore, brachydin B appears to be a promising lead for treating Leishmaniasis and Chagas disease [97,102].

In vivo analysis in *C. elegans* evinced low toxicity of the dimeric compound fukugiside and its anti-virulence potential against *S. pyogenes* [109]. A study with the dimeric flavonoids amentoflavone and robustaflavone demonstrated their ability to reduce the infection by *L. amazonensis* in mice [101].

3. Conclusions

Besides their nutritional value, flavonoid compounds have gained special interest, given the numerous studies that have pointed out their potential in clinics [46,123]. Several researchers have demonstrated the individual or synergistic anti-microbial potential of natural and synthetic flavonoids against drug-resistant fungi [13], bacteria [14], viruses [15] and parasites [16].

Currently, the dimeric flavonoids offer an opportunity for new therapeutic drugs, as proven by the many compounds studied, not only for biological features, but also by toxicity levels [46]. Regarding viruses, amentoflavone and agathisflavone have shown a high spectrum of anti-viral activity against herpes simplex, influenza, dengue, and SARS-CoV-2, with viral enzymes being the main targets of overall compounds [50,61,68,72,73].

In the group of fungi, dimeric flavonoids have more activity towards *C. albicans*, like amentoflavone and proanthocyanidin, and the overall targets are enzymes, biofilm and germinative tube formation [85,89].

Amentoflavone and morelloflavone are compounds with promising effects against *Leishmania* spp. by interacting with enzymes and enhancing antioxidant activity [96,106]. Brachydins discovered in Brazil from the plant *Arrabidaea brachypoda* have strong activity against *Trypanosoma cruzi* by inhibiting the parasite invasion and its intracellular multiplication in host cells [101,102]. In bacteria, many compounds, such as macrophylloflavone, isoginkgetin and ericoside, have shown activity against *Staphylococcus aureus* and *Escherichia coli.* Although some of the mechanisms of action are unknown, major dimeric compounds with antibacterial activity are showing the ability to interfere with nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, and porins in cell membranes [80,105,111].

Future work on the long road to implement the clinical use of these dimeric compounds is needed to clarify their mechanisms of action and toxicity levels. In vitro findings open great possibility for carrying out tests on animal models and clinical trials [46,97,102,118].

Even with several complicated steps, such as natural isolation, synthesis and modifications, these molecules may be important to fight emergent microbial diseases and especially the threat of antimicrobial resistance [41].

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