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# FLAVONOIDS – AN AMAZING GROUP OF COMPOUNDS WITH POTENT ANTIMICROBIAL PROPERTIES

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Antibiotic resistance is spreading all around the world, many infectious diseases are becoming more and more severe and sometimes impossible to treat, therefore new efficient solutions are desperately needed. A possible solution could be represented by natural compounds such as flavonoids - a large and diverse group of plant compounds with variable phenolic structures and diverse biological properties. Flavonoids were used for a long time in traditional medicine for the treatment of infectious diseases due to their antiviral, antibacterial and antifungal activities. Many natural and synthetic flavonoids were found to be more efficient than some drugs used in conventional therapy. Moreover, due to their synergistic effects and various modes of actions, flavonoids represent a reliable solution to fight antibiotic resistance. This review explores the antimicrobial properties of natural and synthetic flavonoids by examining available data in the literature, with special emphasis on their antibacterial activity and mechanism of action.

*Keywords:* flavonoids, antimicrobial, mechanism of action, synergism

## 1. INTRODUCTION

Antibiotic resistance is spreading all around the world, reaching dangerously high levels. In addition, new resistance mechanisms are emerging, affecting our possibilities to treat common infectious diseases and dramatically increasing healthcare costs. Many infectious diseases, such as pneumonia, tuberculosis, gonorrhea and foodborne diseases, are becoming more and more severe and sometimes impossible to treat, because antibiotics are becoming less effective (World Health Organization, 2018). In the USA alone, antibiotic-resistant pathogens associated with hospital acquired infections are causing 99,000 deaths annually. In 2006, about 50,000 Americans died due to pneumonia and sepsis, costing about $8 billion to the US economy [1]. Taking into account this serious problem, the World Health Organization considers antibiotic resistance *one of the biggest threats to global health, food security and development today*. Without

urgent action, we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill (World Health Organization, 2018).

Antibiotics are drugs which target the bacterial cell, often inhibiting the synthesis of proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or disorganizing the cell wall and membrane [2]. Since their discovery in 1940s, the antibiotics were seen as the "wonder drug", a real *magic bullet* that selectively targets the microbes causing the diseases without side effects for the patient. The modern "antibiotic era" came with the optimistic belief that infectious disease will be eradicated due to antibiotics treatment. However, an increased demand for antibiotics across many sectors lead to their enormous and irresponsible use, contributing in short time to the appearance of resistant strains [3].

The antibiotic resistance mechanisms can be divided into two categories. The first ones are represented by intrinsic resistance, which is mainly a characteristic of a particular bacterium, being based on bacteria biological properties. The second ones are related to the acquired resistance, mainly due to the acquisition of resistance genes through mutations or from other pathogenic bacteria. The expression of efflux pump and bacterial biofilm formation is considered to play an important role in the resistance mechanism [4]. In the past, the production of new antibiotics was directly proportional to the development of resistant strains. Nowadays, resistance to an antibiotic develops in a very short time and researchers can't keep up. Hence, antibiotic resistance is a matter of serious concern for humankind [3].

In order to cope with the antibiotic resistance crisis, new solutions are desperately needed. Therefore, great scientific efforts, alongwith great investment in the field of antimicrobials are needed in order to avoid a dangerous public health crisis [5]. Many strategies were employed by researchers to fight back antibiotic resistance. One of them is related to the structural modification of antimicrobial drugs which are ineffective due to microbial resistance. In this way, the lifespan of some antifungal agents such as azoles, antiviral agents – *e.g.* the non-nucleoside reverse transcriptase inhibitors and antibacterial agents including β-lactams and quinolones – was effectively extended. However, with the portfolio of chemotherapeutics currently available, it seems that researchers are getting close to the end game in terms of parent structure alterations [5]. Rational drug design could provide new antimicrobials, but the results obtained up to now showed that the new synthesized compounds were not always effective as antimicrobial drugs. One of the most promising approaches is the development of drugs that target bacterial virulence factors, however, unfortunately, up to now this is not proven completely. In this case, the antimicrobials are not inhibiting the cellular components necessary for growth or viability, but ameliorate infection by interfering with bacterial virulence [6]. Finally, a combination therapy using antibiotics and natural antimicrobial substances, together with the use of new drug delivery systems are also important strategies used to fight antibiotic resistance [4].

Over the past decades, broad screening of natural and synthetic chemical entities for antimicrobial activity represented one of researcher's main concerns as an alternative strategy for the development of novel drugs. Natural compounds were seen as a rich antimicrobial agents source [6]. In this context, plant-derived compounds were considered very attractive for scientists, because plants can be selected for antibacterial testing based on their ethno-medicinal use [7]. In addition, plant-derived drugs remain an important resource, especially in developing countries, to combat diseases. Approximately 60–80% of world's population still relies on traditional medicine for the treatment of common illnesses [8]. Among plant-derived products, flavonoids represent a large and diverse group of compounds used for a long time in traditional medicine for the treatment of infectious diseases [4]. Flavonoids were constantly used in the past for the treatment and prevention of various infectious diseases, such as respiratory, gastrointestinal, urinary and skin infections.

The aim of this review is to explore the antimicrobial properties of natural and synthetic flavonoids by examining available data and reports in the literature. Special emphasis will be put on the antibacterial activity and mechanism of action of flavonoids. Cushnie & Lamb [5,9] and Cazarolli [10] reviews are recommended for the readers who want more information about the antimicrobial properties exhibited by flavonoids. This review article underlines the idea of using natural and synthetic flavonoids as an alternative solution to antibiotics in order to fight back the resistance phenomenon.

Pubmed, Web of Science, Scopus and Science Direct databases were searched for available data using keywords such as natural/synthetic flavonoid/flavones/ flavonols/flavanols/chalcone, antimicrobial/antiviral/antibacterial/antifungal.

## 2. CHEMICAL PROPERTIES AND OCCURRENCE

Flavonoids (Latin word *flavus* = yellow) are a major class of natural compounds with variable phenolic structures, widespread in the plant kingdom. More than 9,000 compounds have been identified in plants and novel compounds are yet to be identified [11]. The name is related to one of their biological functions, flavonoids being responsible for the color of fruits and vegetables (*e.g.*, the red and dark blue color of berries, as well as the orange or yellow color of the citrus fruits) [12]. Flavonoids are secondary metabolites naturally occurring in fruits, vegetables, nuts, seeds, stems and flowers, as well as tea, coffee, red wine, propolis and honey, representing a common constituent of the human diet [5,13].

Flavonoids are considered as derivatives of 2-phenyl-benzo-γ-pyrone. Chemically, the core structure of flavonoids is based upon a C6–C3–C6 skeleton (Fig. 1), in which the carbon atoms are assembled into two benzene rings (A and B), linked *via* a heterocyclic oxygen containing a pyrene ring (C) [12].

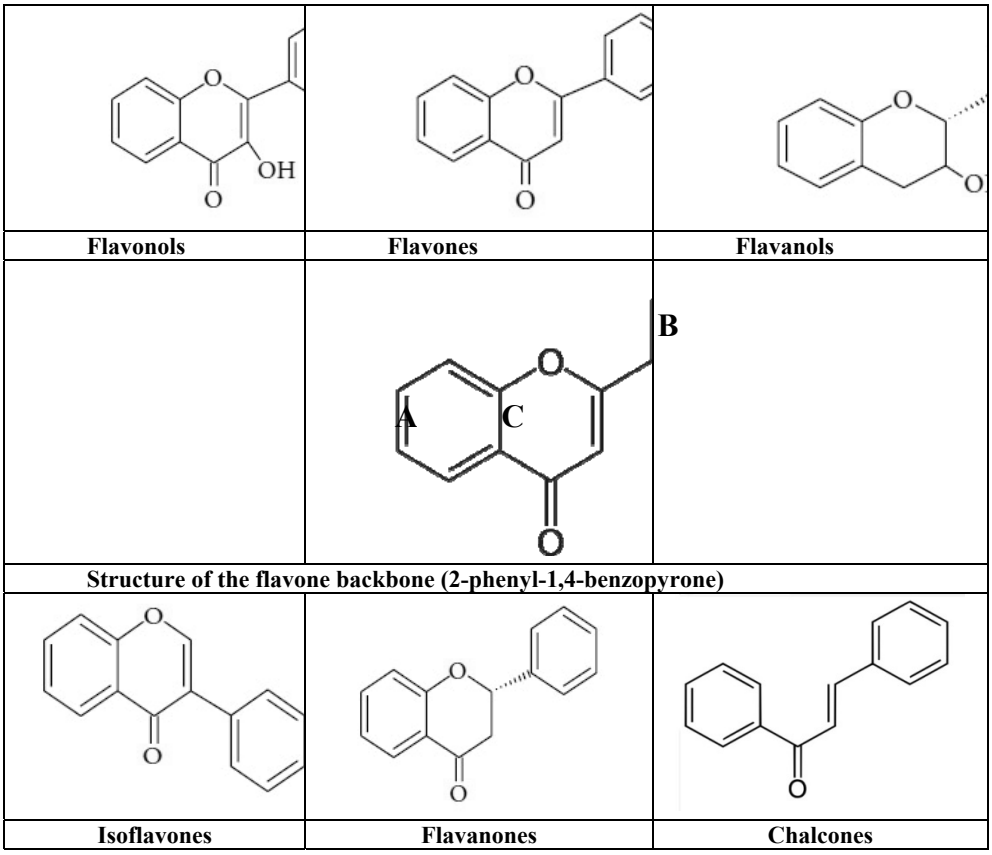


Fig. 1. General structure of different flavonoids classes.

Due to the diversity of their chemical structure, flavonoids are divided, depending on the modifications of the central C-ring, into several classes found in higher plants: flavanols, flavanones, flavonols, isoflavones, flavones and anthocyanins [12]. Non-cyclization of the C ring gives rise to chalcones [13]. Classification of flavonoids is considered a challenge, because some groups – such as chalcones, flavanones and flavanols – are both intermediates in biosynthesis, as wells as end products accumulating in plant tissues, while other classes, such as flavones and flavonols, are identified as end products in biosynthesis [14].

The most abundant flavonoids class present in food is represented by **flavonols**. The main structural particularity is the 3-hydroxy-flavone backbone structure with different positions of the phenolic OH group (Fig. 1). The specific position of the OH group is related to the diversity of this major class of flavonoids, as well as to an increase of the oxidation state [13]. The best known representatives are quercetin (most ubiquitous), kaempferol, myricetin, galangin and fisetin, commonly found in apples, bananas, onions, berries and beverages -

such as red wine, tea and cider [13]. Also, high levels of flavonols were detected in strawberry, spinach and cauliflower [15].

**Flavones** have a very similar structure to flavonols, but with no hydroxylation at the 3-position on the C-ring (Fig. 1). Also, their distribution in plants is limited, compared with flavonols, and their dietary significance is considered to be low. Among the most abundant natural occurring flavones, mention should be made of apigenin and luteolin, while tangeritin, chrysin, baicalein, scutellarein and wogonin are also present [13].

**Isoflavones** differ from flavones in the location of the phenyl group - at C3 rather than at C2 position (Fig. 1). They are known to act as phytoestrogens, due to their ability to bind to estrogen receptor. Although they are very abundant in leguminous species, isoflavones have a very limited distribution in plants. Common isoflavones, such as genistein, daidzein, glycitein, occur in soybeans, but also in black beans and green peas [16].

**Flavanols**, sometimes referred to as flavan-3-ols, are polyphenols with a hydroxyl group at position 3 as well as a fully saturated carbon ring structure – Fig. 1 [13]. Flavan-3-ols are considered structurally the most complex subclass of flavonoids, including simple monomers: catechin and its isomer epicatechin, epicatechin gallate, epigallocatechin gallate, oligomeric and polymeric proanthocyanidins, also known as condensed tannins [17]. They represent the most common flavonoids consumed in the Western diet, being present in various beverages (*e.g.* green tea, wine and beer), whole and processed foods, herbal remedies and supplements. Flavanols affect some quality features of food, such as astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation [16]. Flavan-3-ols are found abundantly in fruits - apricots, sour cherries, grapes, blackberries and apples [16].

**Anthocyanins** are water-soluble plant pigments present in fruits and flower tissues, being responsible for their different colors: red, blue, purple [16]. The most widespread anthocyanin in fruits is cyanidin-3-glucoside. Malvidin glycosides are characteristic for red grapes [18]. Cyanidin and other anthocyanins are present in different berries, providing the fruits with their distinctive and vibrant palate of colors: cranberry, blackberry, blueberry and elderberry [16].

**Flavanones** are mainly represented by naringenin, hesperetin, and eriodictyol, occurring in citrus fruits: grapefruit (naringin), oranges (narirutin), lemon and lime (eriodictyol-7-O-rutinoside). Naringenin is also found in tomatoes [16].

**Chalcones** are aromatic ketones composed of two phenyl rings linked together by a 3-carbon structure, serving as intermediates in the biosynthesis of many other flavonoids – Figure 1 [13]. Licochalcone A and B, found in the *Glycyrrhiza glabra* or *Glycyrrhiza inflata* roots and in carvacrol (oregano, thyme, pepperwort, and wild bergamot) are the most known chalcones.

Flavonoids can be named in three different ways. As Cushnie and Lamb suggested in their remarkable review, the most used names are the trivial ones, indicating sometimes the flavonoid class or plant source. For example, names

ending in ‘inidin’ can denote an anthocyanidin, names ending in ‘etin’ denote a flavonol. Compounds such as tricin and hypolaetin have been extracted from plants belonging to the *Triticum* and *Hypolaena* genera. Other ways to name flavonoids are the semi-systematic manner based on trivial names, such as flavone or chalcone as the parent structure and by giving systematic chemical names, but these are rarely used [5].

Flavonoids are often called bioflavonoids, in relation to their presence in plants and fungi and also in relation to their biological activities. They are present in all plants organs, but their quantitative distribution varies from organ to organ or even from plant to plant, depending on the environmental conditions. Such compounds have a variety of functions in different plant organs [19]. The function of flavonoids in flowers is to provide colors, for attracting plant pollinators [5]. Therefore, some flavonoids are yellow pigments (flavonols, flavones, chalcones), red, violet or blue (anthocyanins), brown (tannins), found not only in flowers, but also in fruits and in other plant organs. Others (colorless flavonones and flavanonols) are copigments. In leaves, flavonoids are involved in promoting plant’s physiological survival, protecting it from biotic factors, such as different microbial pathogens and herbivores or from abiotic factors, such as UV-B radiation, cold, heat, drought, and salinity [19,20]. Also, flavonoids are involved in important physiological processes, such as photosynthesis, energy transfer, the actions of plant growth hormones and growth regulators, control of respiration and photosynthesis, morphogenesis and sex determination [5].

It is widely accepted that natural flavonoids occurring in plant derived-foods are relevant, not only for their organoleptic properties, but also because of their health-promoting effects [12]. Flavonoids were used since ancient times in the traditional medicine of different people for the treatment of many ailments. The large use of flavonoids is related to their diverse biological properties: antioxidant, antitumoral, anti-inflammatory, neuro- and cardio-protective, oestrogenic, antiallergic and antimicrobial.

In the following, we will detail the antimicrobial properties of different natural and synthetic flavonoids.

## 3. ANTIMICROBIAL PROPERTIES

Scientific interest in natural flavonoids has increased in the last decades, mainly due to their recognized impact on human health. Researchers became more interested in knowing how these compounds synthesized by plants can make the human body function better. Even if the existence of flavonoids as pigments in plants has been known for a long time, these compounds were not identified and chemically characterized until the end of the 19th century and early years of the 20th century [10]. The field of flavonoid research recorded a remarkable progress after 1990,

expanding from basic chemistry (isolation and characterization of flavonoids present in plants) to clinical studies in humans. Most likely, the increase of scientific research on flavonoids is related to the great interest in healthy foods and supplements. It was also probably stimulated by the latest studies, which related the presence of flavonoids in fruits and vegetables and the incidence of cancer, stroke and coronary heart diseases [21]. The results of this expanded research on flavonoids can also be found in the field of antimicrobials. Flavonoids are becoming the subject of extended antimicrobial research. More and more structures of flavonoids possessing antifungal, antiviral and antibacterial activity were isolated and identified by many research groups [5]. Thus, new antibacterial agents were developed, such as chrysine, quinine and coumarin derivatives [4].

Flavonoids as natural products were used for centuries in traditional medicine for the treatment of infectious diseases. Their use under the form of propolis and honey is mentioned in the Old Testament and also in the writings of Hippocrates in Ancient Greece for the treatment of sores and ulcers. Many reports attributed the antimicrobial properties of propolis to its high flavonoid content, and especially the presence of galangin and pinocembrin [5]. The most important flavonoids extracted from honey are pinocembrin, pinobanksin and chrysin. Since these flavonoids possess antimicrobial activity, they may be considered as a class of *inhibines* present in honey [22].

Some preparations from the *Tagetes minuta* plant (containing quercetagetin-7-arabinosyl-galactoside) have been reported to treat infectious diseases in Argentina [23]. A root extract from the *Scutellaria baicalensis* medicinal plant has been used in China for thousands of years under the name Huang-Qin. Its application in the treatment of periodontal abscesses and infected oral wounds, diarrhea, disentery, inflammation and respiratory infections is explained by the presence of flavones such as baicalin, wogonoside and wogonin, reported to have various pharmacological functions, including antibacterial and antiviral effects [24].

Extracts of *Tripleurospermum disciforme* are used in Iranian folk medicine as a disinfectant and for treating some diseases, due to their high flavonoid content (apigenin, kaempferol, luteolin, quercetin) [25]. Flower extracts of *Retama raetam* containing licoflavone C and derrone displayed antibacterial activity against Gram-positive and Gram-negative bacteria [19].

Known in China for over 5,000 years, tea was used as an important component of Chinese and Japanese civilizations, being very popular due to its taste and medicinal qualities. Numerous studies revealed a broad spectrum of pharmacological properties of the leaves extracts of *Thea sinensis*, including antibacterial, antifungal and antiviral effects. Tea leaves produce organic compounds such as catechins, responsible for its activities against foodborne and other pathogenic bacteria, bacterial toxins, bacteriophages, pathogenic viruses and fungi [26].

Chamomile (*Matricaria chamomile*) is a herbal medicinal plant used since ancient times across Europe and North America under the form of aqueous and alcoholic extracts obtained from flowers, for treating skin infections caused by

pathogenic bacteria, mouth injuries and respiratory system infections, as well as digestive disorders. Chamomile flowers contain flavonoids such as a glycogen, apigenin, flavone glycoside and lutoline, responsible for its therapeutic effect [27].

The inner bark and leaves of several tree species from the *Cinnamomum* genus are one of the most important and popular spices used not only for cooking but also in traditional and modern medicine. In Ayurvedic medicine, different *Cinnamomum* plant extracts were used in the treatment of diarrhea and also for their antihelminthic, antimycotic, antibacterial and anti-inflammatory effects. Cinnamon has also been traditionally used to treat toothaches and dental or oral problems. Several flavonoid compounds (gossypin, gnaphalin, hesperidin, hibifolin, hypolaetin, oroxindin, and quercetin) have been isolated from *Cinnamomum* extracts [28].

Plants belonging to the *Cistus* species are perennial shrubs frequently used in many traditional medicines throughout the Mediterranean region for their antimicrobial and anti-inflammatory properties. The leaves of all these species secrete resins composed mainly of flavonoids, such as aglycones and glycosides, responsible for the antimicrobial properties against many pathogenic bacteria and fungi [29].

The bitter orange (*Citrus aurantium*) is usually utilized as a flavoring agent for food. The fruits of *C. aurantium* are sources of flavonoid-type compounds, with diverse biological effects, such as antibacterial effects against the food-borne pathogen *Salmonella enterica*, as well as antifungal effects against *Saccharomyces cerevisiae* and *Mucor ramannianus* [30].

*Crataegus* species (hawthorn) have been used in traditional medicine of many European Arabian, Asian and South American countries since ancient times. Decoctions and infusions of leaves, unripe fruits and flowers are used as antidiarrheal and diuretic products. The active flavonoids identified in the extracts might be responsible for the antimicrobial potential against viruses, bacteria and fungi [31].

*Ginkgo biloba* L. is a traditionally important plant cultivated in China, Japan and Korea for its aesthetic and medicinal value. The medicinal parts of *Ginkgo* (fresh or dried leaves, and seeds) are known for their antioxidant, wound healing, neuroprotective and antimicrobial properties. The antibacterial, antifungal, antiviral and antiprotozoan properties of *Ginkgo* can be attributed to its important chemical constituents, such as flavones and flavanols (kaempferol, cvercetol and luteolin derivatives), biflavones (bilobetine, ginkgetin, izginkgetine) and proanthocyanidols [32].

The *Passiflora* genus comprises several species well known for their ethnobotanical uses, especially for their anti-inflammatory, anticancerogenic, antiviral and antibacterial effects. Most of the active components in this plant are C-glycosyl flavones based on apigenin and luteolin. Previous studies revealed that several o-diphenol compounds, in particular the catechin derivatives, are responsible for the observed strong antimicrobial activity [33].

*Tilia cordata* has been used in folk medicine as a sedative for sleep disorders or anxiety since the end of the middle ages. Phytochemical studies have demonstrated that *Tilia* species contain flavonoids, such as rutin, hyperoside, quercitrin and tiliroside, responsible for the antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus epidermis* [34].

Different *Vaccinium* species (*V. myrtillus, V. vitis-idaea, V. macrocarpon*) have been used in phytomedicine for more than 1,000 years. Due to their astringent and antiseptic properties, fruits are recommended in diarrhea or disentery. The fruits are also used to treat oral infections, cough or tuberculosis. The significant antibacterial effect and the additional health benefits of *Vaccinium* fruits are related to their high content of phytochemicals, such as anthocyanins, which are also responsible for their red, purple and blue colours [35].

Wine is a beverage with a complex chemical composition, used since ancient times dating back to the 3rd millennium BC not only as a food, but also as a medicine and solvent for different medicinal preparations. Red wine is a dietary source of phytochemicals, particularly flavonoid compounds, such as flavanols - (epi) catechin, flavonols (myricetinand quercetin) and anthocyanins (malvidin-3-glucoside). The most documented biological properties of red wine are related to the prevention of some diseases, due to its strong antioxidant capacity. Equally, some studies emphasized the antimicrobial, especially the antibacterial activity of red wine. Thus, potential anticaries activity and strong inhibition of biofilm formation, together with antiviral effects, have been reported [36].

Plants synthesize flavonoids in response to microbial infection, therefore it is not surprising that they produce antimicrobial substances against a wide range of microorganisms [37]. In addition, over time, researchers tried to increase the antimicrobial activity of known natural flavonoids by modifying their structure, semisynthetic or synthetic flavonoids thus resulting. The synthetic modification of natural flavonoid structures has been reported starting with 1981 and many such potent antimicrobial agents are known today [6].

**Flavonoids as antivirals**

Antiviral agents inhibit viruses before entering the host cell, impede their multiplication or prevent them from leaving the infected cell [38]. Most of the investigations concerning the antiviral activity of naturally occurring flavonoids have involved the human immunodeficiency virus (HIV). Studies have shown that baicalin inhibits HIV-1 infection and replication [39], while flavone O-glycoside induced inhibition of HIV-1 entry into cells [5]. Biflavonoids such as robustaflavone, amentoflavone, agathis flavones and hinokiflavone were reported to inhibit HIV-1 reverse transcriptase [40]. Morelloflavone and volkseniflavone exhibited moderate to weak activity [38]. Other HIV enzymes (HIV-1 proteinase and HIV-1 integrase) are inhibited by several flavonoids, such as demethylated gardenin A and 3,2-dihydroxyflavone [41] or robinetin, baicalein and quercetin

[42]. Inhibition of integrase is important, as it can prevent the replication of viruses and can be effective in the treatment of AIDS [38]. HIV-1 activation could be prevented by flavonoids chrysin (reported to have one of the highest therapeutic index against HIV-1), acacetin and apigenin [5,43].

The antiviral activity of flavonoids has been reported against other viruses, such as parainfluenza, herpes simplex virus (HSV), adenovirus, and respiratory syncytial virus (RSV) [38]. Quercetin caused a concentration-dependent reduction in the infectivity and reduced intracellular replication of each virus. Flavonoid derivative quercetin-3,7-O-alpha-L-dirhamnoside displayed an antiviral effect towards RNA virus parainfluenza-3 in the range of 8–32 μg/mL of inhibitory concentration, due to its cytopathogenic effect [75]. Hesperetin reduced intracellular replication of the above-mentioned viruses, while catechin inhibited infectivity, but not the replication of RSV and HSV-1 [44]. In the study of Du *et al*. (2003), leachianone G isolated from the root bark of *Morus alba* showed potent antiviral activity (IC(50)=1.6 μg/mL) against herpes simplex type 1 virus (HSV-1) [45]. Three new flavonoids isolated from an ethanol extract of the seeds of *Aesculus chinensis* showed significant antiviral activities against RSV, with IC50 values of 4.5, 6.7, and 4.1 μg/mL, and also against Flu A, with an IC50 of 24.5 μg/mL [46]. Also, three proanthocyanidins from *Pavetta owariensis* have been reported as active against the HSV and coxsackie B viruses [47]. Chrysin and kaempferol, two of the flavonoids found in propolis, inhibit viral replication of HSV, human coronavirus and rotavirus [48]. The same viruses are significantly affected also by flavonol galangin [49]. New flavonoids from *Caesalpinia pulcherrima* were reported as antiviral agents against herpes viruses (HSV-1, HSV-2) and adenoviruses (ADV-3, ADV-8, ADV-11) [50]. The viral inhibitory effects of flavonoids isolated from *Ficus virens* leaves on Coxsackie B4 and hepatitis A virus were reported in 2016 by Orabi and Orabi [51]. Quercetin significantly decreased the viral genome replication, the production of infectious hepatitis C virus particles and the specific infectivity of the newly produced viral particles [52]. Also, quercetin and its derivatives from the Brazilian shrub *Bauhinia longifolia* inhibited the arthropod-borne Mayaro virus production (which causes "Mayaro fever", a disease of medical significance) by more than 90%, displaying a stronger antiviral effect than the licensed antiviral ribavirin [53].

Naturally occurring flavonoids such as quercetin, naringin, hesperetin, and catechin possess a variable spectrum of antiviral activity against certain RNA (RSV, Pf-3, polio) and DNA (HSV-1) viruses acting to inhibit infectivity and/or replication [54]. Apigenin, luteolin, kaempferol, formononetin, and penduletin showed inhibitory effects against Enterovirus 71 in cell cultures [55]. Chrysosplenol B and chrysosplenol C isolated from *Chrysosplenium* plants showed potent antiviral activity, especially against rhinovirus [56].

In a study performed by Lani *et al*. (2016), baicalein, fisetin, and quercetagetin displayed potent inhibition of Chikungunya virus (CHIKV) infection,

a mosquito-transmitted virus that can cause incapacitating arthritis in infected individuals. The results indicated that each of these flavonoids inhibited CHIKV binding to the Vero cells, and displayed potent activity against extracellular CHIKV particles. The mechanism of action is related to CHIKV RNA production and viral protein expression [57].

Some studies were conducted in order to expose the antiviral activities on flavonoids against animal viruses, such as feline calicivirus (FCV) and murine norovirus (MNV). Kaempferol, daidzein, quercetin and fisetin significantly reduced the FCV titer up to 69.76 %. The titer of MNV was reduced up to 51.21 % by fisetin, epicatechin gallate, daidzein and quercetin [58]. Mexican propolis quercetin applied before infection decreased canine distemper virus expression correlated with increased cell viability [59].

As far as synthetic flavonoids are concerned, 6,4-dichloroflavan was reported to show a strong antiviral activity. However, this compound proved unsuccessful in clinical trials [5]. A novel class of thioflavone and flavonoid derivatives were reported by Zhang *et al*. as exhibiting a good antiviral activity against enterovirus 71, coxsackievirus B3 and B6 [60]. Also, substituted homoisoflavonoids showed a marked antiviral activity against Coxsackie virus B1, B3, B4, A9 and echovirus 30 [61]. Some new synthetic 3-hydroxyflavones, 3-acetoxyflavones, and substituted cinnamic and benzoic acid flavon-3-yl esters fluoro-substituted flavones were reported to have a good antiviral activity against picornaviruses (rhinovirus serotype 1B and 14, and poliovirus type 2) [62]. A set of synthetically generated chalcones showed inhibition of viral translation without significantly affecting viral replication in cells infected with hepatitis C virus in a study performed by Mateeva and co-workers (2017) [63].

The mechanisms of action of antiviral flavonoids include inhibition of viral polymerase, binding of viral nucleic acid or viral capsid proteins, inhibition of intracellular replication and of infection processes [5].

**Flavonoids as antifungals**

One of the physiological functions of flavonoids in plants is related to protection against phytopathogenic fungi. Thus it is not surprising, as naturally occurring flavonoids were tested for their antifungal effects against both plant and animal pathogenic fungi. Therefore, some flavonoids were proposed for use in medicine, agriculture and also for food protection.

Many reports concerning flavonoids as antifungals are related to their activity against *Candida albicans*. This situation is easy to understand, if considering the medical importance of this yeast and also the fact that *Candida sp.* are the most commonly strains primarily used in antifungal assays [13]. A remarkable collection of data concerning the anti-*Candida* activity of flavonoids can be found in the excellent review of Seleem and co-workers, published in 2017. Quercetin, myricetin and kaempferol isolated from Chilean propolis inhibited the *in vitro*

growth of *C. albicans* with MIC values ranging between 197-441 µg/mL [64]. A quercetin derivative - quercetin 3-O beta glucoside isolated from *Daucaus littoralis* plants – showed better anti-*Candida* activity, with MIC values as low as 7.8 µg /mL. Rutin and quercitrin isolated from *Duguetia furfuracea*, a plant used in popular medicine, exhibited antifungal activity and synergic effect with fluconazole against C. *krusei, C. tropicalis* and *C. albicans* [65]. Another flavonol – galangin – was found to inhibit the growth of *C. albicans* at a concentration of 31.2 µg/mL [66]. Yoon *et al*. reported the inhibition of *C. albicans* colony formation in the presence of isoflavonol glycosides talosin A isolated from actinobacteria *Kitasatospora kifunensis* at 15 µg/mL [67].

Green tea containing flavanols has been found to have anti-*Candida* activity, resulting in a 90% growth inhibition at MIC of 15.6-250 µg/L [68]. Another known tea, polyphenol-epigallocatechin gallate, was reported to enhance the antifungal activity of antimycotics due to its synergistic effect with miconazole, fluconazole and amphotericin B. The results suggest that flavonoids in combination with conventional antimycotics may be used as an alternative therapeutic strategy to fight the emergence of drug-resistant *Candida* strains [13]. Gallotannin isolated from *Syzygium cordatum* showed antifungal activity at MIC 0.195 mg/mL [69].

A new flavanone isolated from the *Eysenhardtia texana* shrub was reported to possess anti-*Candida* activity [70], as well as a flavan isolated from *Terminalia bellerica* fruit rind [71]. *Swartzia apetala* has been used as an isolation source for pinocembrin, a flavanone which showed activity against nine yeasts of the *Candida* genus [72].

Dihydrokaempferol, a flavane 3-ol compound isolated from the methanol extract of the stem bark of *Commiphora pedunculata*, was reported to be active against *C. albicans* [73]. Also, lupinifolin, isolated from the leaves, stem bark and twigs of *Mundulea sericea* showed antimicrobial activity against *C. albicans* [74].

Some flavones isolated from *Kaempferia parviflora* and *Asterella angusta* inhibited the growth of *C. albicans* at concentrations ranging between 16–512 µg/mL [13]. Baicalein isolated from *Scutellaria biacalensis* inhibits efflux pump and induces appoptosis in *C. albicans* at concentrations as low as 26 µg/mL [75]. Apigenein isolated from propolis also showed antifungal activity, with MIC ranging between 197–441 µg/mL [64]. Glabridin isolated from *Glycyrrhiza glabra* showed a broad-spectrum antifungal activity against several *Candida* species [76]. Isoflavones sedonan A isolated from *Dalea formosa* and dorsmanin extracted from *Dorstenia mannii* inhibited the growth of *C. albicans* and *C. glabrata* at MIC values of 7.6 µg/mL and 64 µg/mL, respectively [13].

Four flavonoid derivatives, scandenone, tiliroside, quercetin-3,7-O-alpha-L-dirhamnoside and kaempferol-3,7-O-alpha-L-dirhamnoside were tested against *C. albicans*, showing a growth inhibition at 1 µg/mL as potent as ketoconazole [77].

Chalcones extracted from *Zuccagnia punctate* showed anti-*Candida* effects through the inhibition of biofilm and germ tube formation [78]. Carvacrol induces *in vitro* apoptosis in several *Candida* species.

Galangin, a flavonol commonly found in propolis, has been shown to have inhibitory activity against many fungi strains, such as *Aspergillus tamarii*, *A. flavus, Cladosporium sphaerospermum, Penicillium digitatum* and *P. italicum* [5]. Rutin isolated from *Solanum palinacanthum* showed antifungal activity against *A. ochraceus* at a MIC value (35 μg/mL) similar to that of benzalkonium chloride, the only fungicide used in Brazil to control such fungus in coffee beans [79]. Romero *et al*. reported the successful inhibition of both mycelial growth and release of ochratoxin A by *A. carbonarius*, suggesting the possibility of their use as plant fungicides, especially against the growth of ochratoxigenic aspergilli [80]. Kaempferol glycoside, isolated from the ethanol extract of *Saussurea lappa* roots, a Himalayan species growing in Kashmir at altitudes from 2,700 to 4,000 m, showed antifungal activity (with MICs ranging from 0.3 to 1.2 nmol/mL) against *Aspergillus niger, A. ochraceus, A. versicolor, A. flavus, Penicilium ochrochloron, P. funiculosum* and *Trichoderma viride* [81]. Trifolin and hyperoside, isolated from *Camptotheca acuminate*, a plant which produces camptothecin, a promising anticancer and antiviral alkaloid, effectively controlled *in vitro* fungal pathogens such as *Alternaria alternata, Epicoccum nigrum, Pestalotia guepinii* and *Fusarium avenaceum* [82].

Flavones isolated from the methanolic extract of *Psoralea corylifolia* seeds exerted a significant antifungal activity against *Trichophyton rubrum, T. mentagrophytes, Epidermophyton floccosum* and *Microsporum gypseum* [83]. Phytopathogens such as *Altemana altemate, Cladosporium oxysporum, Fusarium culmorum* and *F. avenaceum*, as well as human pathogenic *C. albicans, Saccharomyces cerevisiae* and *Trichosporon beigelii* are inhibited by amentoflavones isolated from different plants, including *Taxus baccata* and *Ginkgo biloba* [84]. A new flavone glucoside isolated from the methanol fraction of *Butea monosperma* flowers showed a strong activity against *Fusarium digitatum* and *Penicillum digitatum* at the same concentration of the standard antifungal agent, griseofulvin [85]. Four flavonoids, eriodictyol, homoeriodictyol, dihydroquercetin and luteolin, isolated from the methanol extract of stems and leaves of *Ficus sarmentosa* displayed excellent inhibitory activity against the crop pathogenic fungi *Fusarium graminearum* and *Septoria zeicola* [86]. Ten flavones, two flavanones and three diterpenoids isolated from extracts obtained from the resinous exudates of *Pseudognaphalium cheiranthifolium, P. heterotrichium, P. robustum* and *P. vira vira* plants reduced mycelial growth and showed inhibitory activity against *Botrytis cinerea* [87]. *Rhyzoctonia solani* was the most sensitive strain to the action of flavone isolated from an acetonic extract of *Feijoa sellowiana* fruits (MIC = 62.5 μg/mL) [97].

Flavanones nobiletin, hesperidin and naringin isolated from mature fruits of *Citrus paradisi* and *C. sinensis* were reported by Salas *et al*. (2011) to have antifungal activity against food contaminants fungi, such as *Aspergillus parasiticus, A. avus, Fusarium semitectum* and *Penicillium expansum* [88]. Five flavonoids isolated from the leaves of mango (*Mangifera indica*) reduced the growth of different fungal species (*Alternaria alternata, A. fumigatus*, *A. niger*, *Macrophomina phaseolina* and *P. citrii*) by 63-97% [89].

Orientin, a flavonoid isolated from *Piper solmsianum*, was reported to exhibit a pronounced activity against *Microsporum canis, M. gypseum, Trichophyton mentagrophytes*, *T. rubrum*, with MICs ranging between 7 and 9 μg/mL [90]. An antifungal activity comparable to that of fluconazole against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* was reported for the luteolin, isoorientin and vitegnoside isolated from the ethanol extract of *Vitex negundo* leaves [91].

Hildegardiol, 2-hydroxymaackiain and farrerol, isolated from the root extract of *Hildegardia barteri* were reported by Meragelman and co-workers in 2005 [92].

Flavonoids isolated from a butanolic fraction obtained from *Mentha piperita* leaves reduced *in vitro* the mycelium growth of *Phoma sorghina* and *Fusarium moniliforme* around 72% and 55%, respectively, when used at a concentration of 5 μg/mL [92].

Synthetic flavonoids were also reported as good antifungal agents. Thus, three synthetic dimethoxy flavones showed excellent antifungal activity against *Aspergillus niger, A. flavus, A. fumigatus, Rhizopus* and *C. albicans*, being able to inhibit their growth at a concentration of 5 μg/mL [93]. Flavonoid derivatives, especially bichalcones, were reported by Sagrera *et al*. (2011) to display interesting *in vitro* activity against some human pathogenic fungi, such as *A. niger* [94]. *C. albicans* and *C. krusei* were the most susceptible fungal strains to pyrazoline and hydrazone chalcones derivatives, as shown in a study conducted by Evranos-Aksöz and co-workers in 2015 [95]. A synthetic quercetin bearing a trifluoromethyl group showed a remarkable increase in the antifungal activity against *C. albicans* cultures compared to rutin, the original compound [96]. Some trihydroxyflavone showed a higher fungicidal activity against *Alternaria tenuissima, Cladosporium cladosporioides, Spicellum roseum* and *Trichoderma hamatum* [97].

Concerning the mode of action, flavonoids inhibit fungi growth in many ways. One mechanism is related to the inhibition of efflux pumps, which ultimately results in inducing cell death or apoptosis. Also, flavonoids induce the disruption of the cellular cytoplasmic membrane and affect cell membrane permeability. Cell wall damage was also previously reported to be responsible for the antifungal activity, as well as for the reduction of the number of hyphal cells and germ tubes. Some chalcones have been involved in the inhibition of the exoenzymes responsible for fungal invasion mechanisms [13]. Inhibition of an important virulence factor – biofilm formation – represents another mechanism of action.

**Flavonoids as antibacterials**

A large amount of information about the antibacterial properties exhibited by flavonoids can be found in the literature. The scientific interest in this domain is stimulated by the increase of antibiotic resistance around the world and also by the urgent need to find new effective antibacterial agents. Since flavonoids' mechanisms of action are diverse and yet not overcome by the antibiotic resistant bacterial strains, it is believed that these compounds may be used therapeutically,

to replace conventional antimicrobial drugs or to be applied in combination with antibiotics. In addition, natural flavonoids with antibacterial activity were used for a long time in traditional medicine, as previously discussed in this paper.

The antibacterial activity of naturally occurring flavonoids, as well as that of semisynthetic and synthetic flavonoids, is covered in many reviews, such as that of Cushnie and Lamb (2011) or of Farhadi *et al*. (2018).

Two of the most potent antibacterial natural flavonoids known today are panduratin A, a natural chalcone compound isolated from the rhizome of fingerroot (*Boesenbergia rotunda*) and isobavachalcone, isolated from the twigs of *Dorstenia barteri*. The MIC value against Gram positive and Gram negative bacteria ranged from 0.06-2.0 μg/mL to 0.3–0.6 μg/mL, respectively [6]. Scandenone, an isoflavone obtained from the roots of *Derris scandens*, was also reported as a potent antibacterial flavonoid, with MIC values of 0.5-8 μg/mL against bacteria such as *S. aureus, Bacillus subtilis* and *Enterococcus faecalis*, and 2–32 μg/mL, respectively, against *E. coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii*.

The gancaonin Q and amentoflavone flavones isolated from *Dorstenia* spp. showed a good activity against *Bacillus cereus*, with MIC values of 2.4 and 3 μg/mL, respectively [4]. Higher MICs, of 7.81 μg/mL, were reported by Edziri *et al*. (2012) for licoflavone C isolated from *Retama raetam* flowers, against *E. coli* [98]. The flavone from an acetonic extract of *Feijoa sellowiana* fruits showed a high antibacterial activity against *P. aeruginosa*, *P. vulgaris* and *Proteus mirabilis* (MICs ranging from 1.95 μg/mL to 3.9 μg/mL), and was significantly more active against *Helicobacter pylori* than metronidazole [99]. Artocarpin extracted from the leaves of *Artocarpus anisophyllus* showed good activity against *B. cereus, E. coli* and *Pseudomonas putida*. Another effective bactericide flavone is baicalein, which produced a biofilm formation inhibition effect against *S. aureus*, and also a synergistic effect when combined with cefotaxime [100]. *Glycyrrhiza uralensis* was used as a source for gancaonin G, a flavone with antibacterial activity against vancomycin-resistant *Enterococcus* bacteria, at MIC values of 32 and 64 μg/mL [4].

Artonin I isolated from *Morus mesozygia* inhibited *S. aureus* bacterial efflux pump by 69–89% and increased the susceptibility of the existing antibiotics [101].

Flavonols such as quercetin, myricetrin, galangin, rutin, were reported to present potent antibacterial activities. Quercetin, an ubiquitous flavonoid, is known to exhibit antibacterial effects against bacteria affecting the gastrointestinal, respiratory, urinary, and dermal system [102]. It showed a significant antibacterial activity against resistant bacteria, including methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis*. Also, quercetin isolated from *Croton menyharthii* appeared as active against *Bacillus subtilis*. Wang *et al*. (2018) reported that quercetin significantly decreased the copies of *P. aeruginosa*, *S. enterica* serotype *Typhimurium*, *S. aureus* and *E. coli* from the cecal microbiota of Arbor Acre broiler chickens, and exerted a bacteriostatic effect against all four bacteria; the bacteriostatic effect of quercetin was stronger on Gram-positive than on Gram-

negative bacteria [103]. Quercetin-derived oxidation products isolated from a water extract of onion (*Allium cepa*) skin showed antibacterial activity against *Helicobacter pylori* and MRSA strains [104]. Quercetin extracted from lotus leaves extracts may be responsible for the antibacterial activity against peridontitis bacteria such as *Actinobacillus actinomycetemcomitans*, *Actinomyces viscosus*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Actinomyces naeslundii* [105]. Basile *et al*. reported in 2000 a high antibacterial activity of quercetin and rutin extracted from *Castanea sativa* leaves against different Gram-positive and Gram-negative bacteria, with MIC value ranges of 64–256 μg/mL and MBC values between 256-512 μg/mL [106].

Some flavonols exerted higher antibacterial effects compared with conventional antibiotics. For instance, piliostigmol from *Piliostigma reticulatum* showed a three times stronger antibacterial effect against *E. coli* than amoxicillin at MIC – 2.57 μg/mL [4]. Lower MICs values (0.5–1 μg/μL) were reported for galangin against Gram-positive bacteria [107]. In fact, galangin is a well-known antibacterial agent, a major constituent of medicines propolis and *Helichrysum aureonitens* traditionally used for a long time in South Africa to treat infections [4]. It was tested against 16 strains of 4-quinolone resistant *S. aureus* and it was determined that galangin had a MIC of 50 μg/mL against these strains [108]. Also, galangin was able to reverse bacterial resistance to conventional β-lactam antibiotics against penicillin-resistant *S. aureus* when combined with quercetin and baicalein [4]. Recent studies showed that galangin could significantly suppress the vancomycin-intermediate *S. aureus* growth at concentrations of 4, 8 and 16 μg/mL. The bacteriostatic effect is related to the significant inhibition of murein hydrolase genes expression (*atl, lytM,* and *lytN*) and of their regulatory genes (*cidR, cidA* and *cidB*) [109]. Also, galangin was reported to cause aggregation of bacterial cells by using the cytoplasmic membrane as a target site for this compound's activity [110]. Morin, which was tested against three bacterial strains: *E. coli*, *K. pneumoniae*, *S. aureus*, was found to be an effective inhibitor of strains' growth [111]. Also, in combination with different antibiotics, such as β-lactams, it decreased the bacterial resistance and improved the efficacy of conventional drugs.

Flavanones are also known for their important antibacterial properties. Prenylated flavanones from *Paulownia tomentosa* fruits showed a strong *in vitro* antibacterial against *B. cereus, B. subtilis, Enterococcus faecalis, Listeria monocytogenes* and *S. aureus*, with MIC values of 2–4 μg/mL [112]. Flavanones such as 3′-Omethyldiplacol and mimulone from the same *P. tomentosa* fruits displayed promising antibacterial activities when used alone or in combination with conventional antibiotics against MRSA (MIC 2–16 μg/mL) [113]. Two flavanones isolated from a methanol–dichloromethane extract obtained from the aerial parts of *Eysenhardtia texana* showed important antibacterial and antifungal activity [70]. Extracts from *Sophora exigua* containing flavanones inhibited the growth of MRSA strains at concentrations of 1.56–6.25 μg/mL [114]. Astilbin, a bioactive flavanone extracted from the leaves of *Harungana madagascariensis* showed

antibacterial activity against the representative skin microflora of the armpit and feet, with MIC and MBC ranging from 25 to 250 μg/mL and 100 to 750 μg/mL, respectively [115]. Popova *et al*. (2005) reported Turkish propolis as a source of flavanones with antibacterial effect against *S. aureus* and *E. coli* [116]. Pinocembrin isolated from *Combretum apiculatum* showed activity against *S. aureus* with MIC of 12.5 μg/mL. Also, pinocembrin from the leaves of *Cryptocarya chinensis* was potent against *Mycobacterium tuberculosis* at a MIC value of 3.5 μg/mL [4]. 6,8-diprenyleriodictyol obtained from *Dorstenia* species reduced bacterial cell density and caused lysis of *S. aureus*, as shown in a study performed by Dzoyem *et al*. (2013) [117]. Lupinifolin from the *Mundulea sericea* has been reported to have significant antibacterial activity against *S. aureus*, at a minimum inhibitory quantity value of 0.5 μg [4]. Sophoraflavanone from *Sophora flavescens* is considered a compound with potent antibacterial activity against *S. epidermidis, S. aureus* (including MRSA) and *B. subtilis*, with MIC values ranging from 3.1 to 12.5 μg/mL [118,119]. Flavanone macatrichocarpins from the acetone extract of the *Macaranga trichocarpa* leaves were found to be moderate antibacterial agents, with the lowest MIC value of 26.5 μM against *B. subtilis* [120]. Six flavanones isolated from *Galium fissurense*, *Viscum album* and *Cirsium hypoleucum* showed activity against extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* at concentrations of 32 – 64 μg/mL [121].

Flavane 3-ols compounds are well known for their antibacterial effects. Thus, conrauiflavonol isolated from *Ficus conraui* showed activity against *E. coli* with MIC values of 64 μg/mL [122]. In a study of Tajuddeen *et al*. (2016), dihydrokaempferol isolated from the methanol extract of the stem bark of *Commiphora pedunculata* was active against *E. coli, S. aureus, P. aeruginosa* and *C. albicans*, with minimum inhibitory concentration – as low as 6.25 μg/mL [73]. Ericoside isolated from the ethanol extract of the whole plant of *Erica mannii* displayed moderate activity against the resistant *E. coli* AG100 with a MIC of 64 μg/mL [123]. Lupinifolin, isolated from the leaves, stem bark and twigs of *Mundulea sericea*, showed antimicrobial activities against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* [74]. *Cistus laurifolius* is the isolation source of quercetin 3-O-methyl ether, a compound with anti-*Helicobacter pylori* activity at concentration of 3.9 μg/mL [124]. Delgado-Adámez *et al*. (2012) reported the antibacterial effect of flavanols isolated from leaf extracts of different *Prunus salicina* cultivars against *Listeria innocua* and *E. coli* [125].

Chalcones represent another class of phenolic compounds with important antibacterial activity. The dichloromethane extract of the leaves of *Myrica serrata* inhibits the growth of *B. subtilis* and *E. coli* [126]. Chalcones isolated from the plants of the *Dorstenia* genus showed bactericidal and bacteriolysis activities against *S. aureus*, with MIC values of 1-8 μg/mL [117]. Antibacterial and synergistic activities of prenylated chalcone isolated from the roots of *Sophora flavescens* were reported by Lee *et al*. (2010) [127]. Wang *et al*. (2015) reported the antibacterial activity of licochalcone A and licochalcone E, isolated from the

root of *Glycyrrhiza glabra* by decreasing expression of bacterial genes, inhibiting bacterial growth and reducing the production of bacterial toxin [4]. 3–Hydroxychalcone exhibited antibiofilm activity against *Haemophilus influenzae*, its mean minimum biofilm inhibitory concentration (MBIC50) being 16 µg/mL, six fold more active than the reference drug azithromycin [128]. A prenylated chalcone isolated from *Elatostema parasiticum* inhibited the growth of *S. aureus* and *B. subtilis* with MIC values of 7.8 µg/mL and 1.95 µg/mL, respectively. 2′,4′,4-trihydroxy-3,6′-dimethoxychalchone isolated from *Piper delineatum* displayed a potent quorum sensing inhibitory activity against *Vibrio harveyi* through inhibition of biofilm formation [4]. Chalcone and dihydrochalcone compounds from *Uvaria chamae* roots (commonly used for the treatment of various infections in south Benin) were highly active against multidrug-resistant Gram-positive cocci, this activity being more extensively compared to that of conventional antibiotics [129].

The natural flavonoids with most potent antibacterial activity were used as models to synthetize new derivatives. The main goal was to increase the biological effects and to obtain new efficient antibacterial agents. Synthetic flavonoids can modulate important cellular functions, affecting virulence by inhibiting the efflux pumps-transport proteins involved in the extrusion of antibiotics from the cells. In this way, the antibiotic resistance mechanisms can be avoided [130]. Many authors reported the antibacterial activity of synthetic flavonoids. Here are some examples of their works.

Some analogues of chrysin, a natural product obtained from *Oroxylum indicum*, were screened for their antibacterial activity against a panel of susceptible and resistant Gram-positive and Gram-negative organisms. They displayed a significant activity, as compared to their parent compound chrysin [131]. A series of Oroxylin A derivatives, prepared by alkylation and condensation, showed good activity against *B. sphaericus* and *Chromobacterium violaceum* with MIC of 6.25 µg/mL [130]. Substituted flavones, 4-thioflavones and 4-iminoflavones were found to be active against *E. coli, B. subtilis, S. flexnari, S. aureus, S. typhi* and *P. aeruginosa*. The compounds with substituents like F, OMe and $NO_2$ at 4'–position in ring-B showed enhanced activity, while the presence of electronegative groups in the studied compounds showed a direct relationship with the antibacterial activity [132].

Zhang *et al*. (2016) reported that a series of 5,7-dihydroxyflavanone derivatives, especially the halogenated ones, exhibited a good antimicrobial activity against Gram-positive and Gram-negative bacteria (*e.g*., *Vibrio cholera*) [133]. Fowler *et al*. (2011) identified the synthetic molecule 4 –chloro-flavanone as a potent antimicrobial compound, with a MIC value of 70 µg/mL against *E. coli*, when combined with the Phe-Arg-ß-naphthylamide inhibitor [134]. Starting from abyssinone II and olympicin A, several chemically modified flavonoids were synthesized. They showed good antibacterial activities against *Mycobacterium tuberculosis*, but also against Gram-positive pathogens, including MRSA, with a MIC value as low as 0.39 µg/mL [135].

Naringin derivatives were presented as promising antimicrobial agents, due to their high antilisterial and antistaphylococcal activity [136]. 7-O-butyl naringenin showed high anti-MRSA, with a MIC value of 0.625 mM, thus being more effective than quercetin and naringenin against MRSA [39].

The antibacterial effect of three newly-synthesized chalcones against clinical isolates of MRSA, and their synergism with β-lactam and non- β-lactam antibiotics were assessed by Bozic *et al*. in 2014. The authors showed significant anti-MRSA activity with MIC values between 25-200 μg/mL. The observed synergism with antibiotics demonstrated that chalcones significantly enhanced the efficacy of ciprofloxacin, gentamicin and trimethoprim/sulphamethoxazole [137]. A series of chalcone derivatives that mimics the essential properties of cationic antimicrobial peptides showed good bactericidal activity against both Gram-positive and Gram-negative bacteria, including the drug-resistant species MRSA, KPC and NDM [138]. A novel bi-functional chalcone, a mannich base derivative of isoliquiritigenin, inhibits the multi-drug resistant *S. aureus* and potentiates the activity of norfloxacin. In addition to its anti-staphylococcal activity, the chalcone derivative inhibits the multidrug NorA efflux pump [139]. The bacteriostatic action of synthetic trihydroxylated chalcones against *E. coli*, at MIC values of 46 μg/mL and 122 μg/mL, was presented by Alvarez *et al*. (2004) [140].

The synthetic molecule 4-chloro-flavanone is reported as a potent antimicrobial compound, with a MIC value of 70 μg/mL in *E. coli* when combined with the Phe-Arg-ß-naphthylamide inhibitor [134].

Since the year 2014, the research group of Birsa and Stefan reported the antibacterial activity of some dithiocarbamic esters bearing a flavanone backbone, as well as of their corresponding 1,3 – dithiolium salts. Such 1,3 – dithiolium tricyclic flavonoids tested against *S. aureus* and *E. coli* displayed good inhibitory activity against both Gram-positive and Gram-negative pathogens at MICs of 0.24 μg/mL and 3.9 μg/mL, respectively [141]. Further on, the authors showed that the tested compounds exhibited significantly enhanced antibacterial activities, 32 to 72-fold more active than of other synthetic flavonoids. Bactericidal activity was recorded at concentrations ranging from 0.48 to 15.62 μg/mL. At double MICs, all *E. coli* and *K. pneumoniae* cells were killed within 1 h. Also, tricyclic flavonoids presented a good anti-biofilm activity. The proposed mechanism of action is related to the impairment of cell membrane integrity [142,143].

One of the strategies used to fight antibiotic resistance is related to the combined therapy. In this case, one or more antibiotics are combined with different natural or synthetic compounds in order to increase their activity against resistant microorganism. Flavonoids were reported to have a synergistic effect in combination with antibacterial or antifungal drugs, being therefore proposed as a therapeutical alternative. There have been many recent reports about flavonoids which may increase the activity of antibiotics, however, from all flavonoids reported to have synergistic activity, flavan-3-ols received most attention [6]. Synergy between naturally occurring flavane-3-ols and antibiotic agents was

reported by An *et al*. (2011). They showed that flavanonol rhamnoside presents a significant synergistic effect with antibiotics including ceftazidime and levofloxacin against *S. aureus* [144]. Also, Navrátilová *et al*. (2016) evaluated the antibacterial activity of 3′-O-methyldiplacol in combination with oxacillin and proved that this combination had a synergistic effect against MRSA strains [113].

Bakar *et al*. (2012) tested the *in vitro* activity of flavones in combination with vancomycin and oxacillin against vancomycin-intermediate *S. aureus* (a multidrug-resistant bacterium) and evidenced synergism with fractional inhibitory concentration index values of 0.094 and 0.126, respectively. A synergistic activity against *S. aureus* through the inhibition of the NorA efflux protein was also evidenced in a study of Qiu *et al*. (2016), where diosmetin and alpinumisoflavone from *Sophora moorcroftiana* were used in combination with ciprofloxacin and baicalin [4].

Four new quercetin-derived oxidation products and lunularin-4-O-β-d-glucoside isolated from a water extract of onion (*Allium cepa*) skin increased the susceptibility of MRSA to β-lactams [104]. Synergism has been demonstrated between sophoraflavanone B and antibiotics including ampicillin, oxacillin, and gentamicin against MRSA strains. Also, sophoraflavanone G from *Sophora flavescens* potentiated the effect of ampicillin or oxacillin against MRSA infections [119]. THIPMC extracted from the plants of the *Dorstenia* genus was reported to be more active against the tested bacteria, when combined with ampicillin or gentamicin than when used alone [127].

In order to explain the antibacterial properties of flavonoids, various **mechanisms of action** have been proposed. Maybe, one of the most cited mechanisms of action is related to cellular membrane impairment. Quercetin damages the cell walls and membranes of *E. coli* and *S. aureus* and significantly increases the activity of ATP and extracellular alkaline phosphatase and β-galactosidase [103]. Kaempferol showed bacterial cell disruption through interaction with the polar head-group of a membrane model [4]. Baicalein affects bacterial *S. aureus* membrane's penetrability, inhibits protein synthesis and influences SDH, MDH and DNA topoisomerase I and II activities in exerting its antibacterial functions [100]. Dzoyem *et al*. (2013) reported that 6, 8-diprenyleriodictyol from *Dorstenia* species deactivated *S. aureus via* membrane depolarization and inhibition of DNA, RNA, and protein synthesis [4]. Soybean isoflavone was reported to increase the permeability on *S. aureus* membranes for DAPI and to completely inhibit topoisomerase I and topoisomerase II at a concentration of 6.4 mg/mL [145].

Inhibition of the bacterial efflux pump and increase in the susceptibility of the existing antibiotics are also responsible for the antibacterial effect of some flavonoids, such as baicalein, artonin I, or chalcones.

Flavones form a complex with the different extracellular cell wall components, such as proteins, thus inhibiting microbial adhesion and growth [4]. Interaction with the cell wall by reducing D-alanylation of the cell wall teichoic

acid was also reported for galloyl flavan-3-ols, as a manner to modulate β-lactam resistance [6].

Another proposed mechanism is represented by the inhibition of bacterial enzymes (such as tyrosyl-tRNA synthetase). Kaempferol-3-rutinoside isolated from *Sophora japonica* flowers inhibited sortase A from *S. mutans*, which plays a key role in the adhesion to and invasion of hosts [4]. 3,6-Dihydroxyflavone was reported to inhibit β-ketoacyl acyl carrier protein synthase I and III, with a MIC value of 512 μg/mL [146].

Baicalein is able to downregulate the quorum-sensing system regulators agrA, RNAIII and sarA, and also the gene expression of intercellular adhesin (ica) in *S. aureus* biofilm producer cells [100].

The antimicrobial activity of flavonoids could be related to their interference with some virulence factors, such as biofilm formation. Thus, baicalein inhibited *S. aureus* biofilm formation and the quorum sensing system *in vitro* [100]. Biofilm eradication was also reported for rutin and myricetin which, at a concentration of 50 μg/mL, reduced biofilms of foodborne pathogens (*E. coli* and *S. aureus*) [4]. Another virulence factor targeted by flavonoids such as polymerised catechin, isoflavone genistein, genistein, is neutralisation of bacterial toxins.

## 4. CONCLUSIONS

Many studies reported the antimicrobial properties of natural and synthetic flavonoids, emphasizing the great scientific interest in this topic. This group of polyphenolic compounds presents potential antiviral, antifungal and antibacterial activity *via* multiple modes of action. In addition to their biological activity, flavonoids exhibit synergistic effects with antibiotics, which evidences their huge potential to be used in combination therapy with the currently used drugs. Based on their synergism and multiple mechanisms of action, flavonoids could be a reliable solution to fight back antibiotic resistance phenomena. However, we must take into consideration that the great majority of the results presented so far in the literature concerning the antimicrobial potential of flavonoids was generated *via in vitro* assays. Therefore, more clinical studies involving flavonoids should be performed in the future for a better understanding of their complex biological activities.

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