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Structure–Activity Relationship of Flavonoids on Their Anti-*Escherichia coli* Activity and Inhibition of DNA Gyrase

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ABSTRACT: Flavonoids are potential sources of natural preservatives. The inhibitory activities of three polymethoxylated flavones (PMFs), three flavones, and four flavonols against *Escherichia coli* were determined using the microbroth dilution method. Flavonoid inhibitory activities against DNA gyrase from *E. coli* were estimated by DNA supercoiling. Kaempferol exhibited the greatest antibacterial activity [minimal inhibitory concentration (MIC) = 25 μg/mL], while nobiletin showed the lowest activity (MIC = 177 μg/mL). A good correlation was found between the pIC_{50} values and the corresponding pMIC values for the purified DNA gyrase (r = 0.9582). The structure–activity relationship analysis suggests that, for a good inhibitory effect, the hydroxyl group substitution at C-5 in the A ring and C-4′ in the B ring and the methoxyl group substitution at C-3 and C-8 in the A ring are essential. The presence of the hydroxyl group at C-6 in the A ring, C-3′ and C-5′ in the B ring, and C-3 in the C ring and the methoxyl group at C-3′ in the B ring greatly reduced inhibition of bacteria. These findings provide a theoretical basis for the development of high-bioactive and low-toxicity natural preservatives.

KEYWORDS: *Flavonoids, DNA gyrase, Escherichia coli, antibacterial, structure–activity relationships*

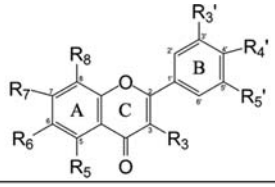
INTRODUCTION

A recent trend in food processing is to limit use of synthetic preservatives, because consumers are increasingly trying to avoid such foods. This is reflected by the food industries' growing interest in developing high-quality products with natural compounds exhibiting antimicrobial activity[1] and subsequent increased research in this area. Flavonoids are secondary plant metabolites, including the following commonly occurring polyphenols: flavanones, flavones, anthocyanins, flavonols, and flavan-3-ols. Flavonoids are well-documented for their biological effects, including anticancer, antiviral, antimutagenic, and anti-inflammatory activities.[2] There is also evidence suggesting that dietary flavonoids can influence gastrointestinal bacterial populations.[3]

The flavones apigenin and luteolin demonstrated selective toxicity to *Staphylococcus aureus*.[4] The flavonol galangin exhibited good antibacterial activity against 17 strains of 4-quinolone-resistant *S. aureus*.[5] Another important flavonoid subclass, polymethoxylated flavones (PMFs), is found in citrus peel. PMFs are a general term for flavones bearing four or more methoxyl groups on their basic benzo-γ-pyrone (15 carbon, C-6–C-3–C-6) skeleton with a carbonyl group at the C-4 position.[6] PMFs have been shown to possess a broad spectrum of biological activity, including antibacterial, antifungal, and antiviral activities.[7−9] PMFs isolated from *Citrus* spp. peels demonstrated antimicrobial activity against *Escherichia coli* and *S. aureus*, especially *Microsporum canis* and *Trichophyton mentagrophytes*.[10]

Flavonoids show potential as natural antimicrobial agents in the food industriy. However, different flavonoids exhibit different biological activity based on substituent groups within their chemical structure (Figure 1). Generally, antimicrobial agents exert an inhibition effect by three mechanisms, including (a) inhibition of nucleic acid synthesis, (b) inhibition of cytoplasmic membrane function, and (c) inhibition of energy metabolism.[11−15] It has been speculated that the mechanism of flavonoid inhibition is based on interaction with DNA. Ohemeng et al. demonstrated that quercetin and apigenin could inhibit DNA gyrase from *E. coli*.[16]

However, the antimicrobial mechanism of flavonoids and how the number and positions of substituted methoxyl and hydroxyl groups affect antibacterial activity remain to be



Flavonoids	R3	R5	R6	R7	R8	R3'	R4'	R5'
Tangeritin	H	OMe	OMe	OMe	OMe	H	OMe	H
5,6,7,4'-tetramethoxyflavone	H	OMe	OMe	OMe	H	H	OMe	H
nobiletin	H	OMe	OMe	OMe	OMe	OMe	OMe	H
chrysin	H	OH	H	OH	H	H	H	H
galangin	OH	OH	H	OH	H	H	H	H
quercetin	OH	OH	H	OH	H	OH	OH	H
baicalein	H	OH	OH	OH	H	H	H	H
luteolin	H	OH	H	OH	H	OH	OH	H
kaempferol	OH	OH	H	OH	H	H	OH	H
myricetin	OH	OH	H	OH	H	OH	OH	OH

Figure 1. Structures of the flavonoids, with rings named and positions numbered.

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clarified. In the present study, we investigated the antibacterial mechanism of flavonoids against *E. coli* by inhibition assay of DNA gyrase from *E. coli* to reveal their structure−activity relationships. The research provides a theoretical basis for the development of high-bioactive and low-toxicity natural preservatives.

MATERIALS AND METHODS

Chemicals. Most of the standard flavonoid compounds were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China) (purity > 98%), including baicalein (5,6,7-trihydroxyflavone), chrysin (5,7-dihydroxyflavone), galangin (3,5,7-trihydroxyflavone), kaempferol [3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one], luteolin [2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-chromenone], myricetin [3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromenone], and quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one]. Tangeritin (5,6,7,8,4′-pentamethoxyflavone) and nobiletin (5,6,7,8,3′,4′-hetramethoxyflavone) were purchased from National Institutes for Food and Drug Control (Beijing, China) (purity > 98%). 5,6,7,4′-Tetramethoxyflavone was purchased from ChromaDex Corp. (Irvine, CA) (purity > 98%). The flavonoids were dissolved in a small volume of dimethyl sulfoxide (DMSO), and the solutions were diluted with water to a final concentration of 1% DMSO. All other chemicals were of analytical grade.

Bacterial Strains and Plasmids. *E. coli* JM109 and *E. coli* DH5α were used as hosts for cloning purposes, and *E. coli* BL21 (DE3) was used for protein expression. The pGEM-T Easy Vector Systems II (Promega Biotech Co., Ltd., Beijing, China) was used to clone the amplified DNA fragments. The pET-28a(+) plasmid DNA (Novagen, Merck China) and pGEX-6P-1GST expression vector (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) were used to construct vectors for overexpression of *E. coli* GyrA and GyrB proteins. Relaxed pBR322 was purchased from TaKaRa (Dalian, China).

Cloning, Protein Expression, and Purification. The gyrA and gyrB genes encoding the A and B subunits of DNA gyrase, respectively, were identified within *E. coli* genomic sequences. The polymerase chain reaction (PCR) was used to amplify each of these open reading frames from *E. coli* genomic DNA, using the following primer pairs: 5′-CCGGAATTCATGAGCGACCTTGCGAGA-GAAATTAC-3′ (forward, underlined bases correspond to the *Eco*RI site) and 5′-CCGCTCGAGTTATTCTTCTTCTGGCTCGTCGT-CAAC-3′ (reverse, underlined bases correspond to the *Xho*I site) for gyrA and 5′-CCGGAATTCATGAGCGACCTTGCGAGAGAAAT-TAC-3′ (forward, underlined bases correspond to the *Bam*HI site) and 5′-CCGCTCGAGTTATTCTTCTTCTGGCTCGTCGTCAAC-3′ (reverse, underlined bases correspond to the *Xho*I site) for gyrB. Genomic DNA was isolated from an *E. coli* DH5α culture grown in Lysogeny broth at 37 °C using the Genomic DNA Preparation Kit for bacteria according to the instructions of the manufacturer (BioTeke Corporation, Beijing, China). The amplification conditions were as follows: for gyrA, 2 min of denaturation at 95 °C, 30 amplification cycles of 1 min of denaturation at 94 °C, 20 s of annealing at 54 °C, and 1 min of extension at 72 °C, with a final extension step for 5 min of extension at 72 °C; for gyrB, 2 min of denaturation at 95 °C, 30 amplification cycles of 1 min of denaturation at 94 °C, 20 s of annealing at 54 °C, and 1 min and 20 s of extension at 72 °C, with a final extension step for 5 min of extension at 72 °C. Amplified DNA products were resolved by electrophoresis on 1% agarose gels containing ethidium bromide. The PCR product purified using a PCR Purification Kit (BioTeke Corporation, Beijing, China) were ligated into pGEM-T Easy Vector and transformed into *E. coli* JM109 competent cells, according to the instructions of the manufacturer.

Recombinant plasmids were recovered from the white colonies and digested with *Eco*RI and *Xho*I (gyrA) and *Bam*HI and *Xho*I (gyrB), and the DNA fragments obtained were ligated into *Eco*RI-*Xho*I-cut pET-28a(+) and *Bam*HI-*Xho*I-cut pGEX-6P-1, respectively, and transformed into *E. coli* DH5α. The recombinant plasmid carrying the gyrA gene of *E. coli* (pET-28-GA) and that carrying the gyrB gene of *E. coli* (pGEX-GB) were separately transformed by electroporation into *E. coli* BL21 (DE3). Three different BL21 clones were grown separately at 37 °C in 100 mL of Luria−Bertani (LB) medium containing the selective antibiotic at 200 revolutions/min overnight. The clone subcultures (1 mL) were then mixed and used to inoculate 50 mL of LB medium containing the selective antibiotic at 200 revolutions/min until the optical density at 600 nm reached 0.4−0.6. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM, and growth was continued for a further 8 h at 18 °C. The induced cells were then recovered by centrifugation at 12000 revolutions/min for 1 min at 4 °C, washed with a buffer of 20 mM Tris-HCl (pH 7.9), 500 mM NaCl, and 60 mM imidazole (Novagen), and stored as a pellet at −80 °C.

Frozen cell pellets were initially washed 2 times with 20 mL of buffer and suspended in 50 mL of wash buffer. After disputation by a high-pressure cell disrupter (Constant Systems, U.K.) and centrifugation at 13000 revolutions/min for 40 min at 4 °C (Beckman, Germany), the suspension containing the His-tagged GyrA and GST-tagged GyrB was purified following the instructions of the manufacturer (Novagen, Merck China, and GST•Bind Purification Kit, GE Healthcare Bio-Sciences Corp., Piscataway, NJ). The GyrA and GyrB proteins were then flash-frozen in aliquots in liquid nitrogen and stored at −80 °C. The protein fractions were examined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE).

DNA Supercoiling Assays. The DNA gyrase supercoiling activity was assessed by measuring the conversion of relaxed plasmid pBR322 DNA to the supercoiled form, as described previously.[17,18] Supercoiling assays were carried out in 30 μL reaction mixtures containing the DNA gyrase assay buffer [35 mM Tris-HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 2 mM dithiothreitol, 1.8 mM spermidine, 1 mM ATP, 6.5% glycerol, and 0.1 mg of bovine serum albumin (BSA)/mL], 150 ng of relaxed pBR322, and 2−3 μL of purified DNA gyrase (GA/GB = 1:1). The mixture was incubated for 30 min at 37 °C. The reaction was stopped by the addition of 50% glycerol containing 0.5% bromophenol blue, and the total reaction mixture was subjected to electrophoresis on 1% agarose gel in TAE buffer [tris(hydroxymethyl)aminomethane (Tris)/acetic acid/ethylenediaminetetraacetic acid (EDTA) at pH 8.0]. After a run of 3 h at 60 V, the gel was stained with ethidium bromide (0.7 μg/mL). Supercoiling activity was assessed by tracing the brightness of the bands corresponding to the supercoiled pBR322 DNA, using a Densylab densitometer (Bio-Rad). A total of 1 unit (U) of enzyme activity was defined as the amount of DNA gyrase that converted 150 ng of relaxed pBR322 DNA to the supercoiled form in 30 min.

Inhibition of DNA Gyrase Supercoiling Activity. Inhibition of the supercoiling activity of the purified DNA gyrase was performed using the method by Staudenbauer and Orr,[19] with minor modifications. In brief, a reaction mixture in the gyrase assay buffer (30 μL), containing 150 ng of relaxed pBR322 DNA, 1 U of purified DNA gyrase, and a serial 2-fold dilution of the flavonoid, was incubated as described above. The inhibitory effect of flavonoid on DNA gyrase was assessed by determining the concentration of flavonoid required to inhibit 50% of the supercoiling activity of the enzyme (IC_{50}). IC_{50} values reported are averages from at least three separate experiments.

Antibacterial Activity. The *in vitro* antibacterial activity was measured as MIC_{50}, which is defined as the concentration that inhibits the growth of 50% of organisms. The MIC_{50} of flavonoids was determined by the microbroth dilution method performed in 96-well microplates as described by Mandalari et al.[20] and Wiegand et al.,[21] with a minor modification. *E. coli* ATCC 25922 used for the biocidal test was provided by the China Center of Industrial Culture Collection. Bacterial strains were maintained on Mueller Hinton Agar, and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 mL of Mueller−Hinton (MH) broth and incubated overnight at 37 °C with shaking (150 revolutions/min). The turbidity of the culture was adjusted with sterile saline solution to match the 0.5 McFarland standard, 1×10^8 colony forming units (CFU)/mL. Then, bacteria were diluted with sterile MH broth (1 mL of bacteria/50 mL of MH). A total of 100 μL of bacterial culture (1×10^8 CFU/mL)

were added to each well. Stock solutions of flavonoids were prepared in DMSO. A total of 100 μL of each dissolved flavonoid was 2-fold serially diluted with sterile distilled water into a 96-well microplate in triplicate. A similar 2-fold serial dilution of neomycin (Sigma) (0.1 mg/mL) was used as a positive control, while bacteria-free broth, distilled water, and DMSO (maximum of 2.5%, v/v) acted as negative controls. The plates were covered and incubated with shaking for 16 h at 37 °C and 220 revolutions/min. Bacterial growth was examined by measuring solution optical density (Multiskan Spectrum, Thermo). The inhibition ratio (%) was calculated as follows: percent inhibition = [(absorbance of the control − absorbance of the test sample)/absorbance of the control] × 100.

Statistical Analysis. All determinations were carried out in triplicate. Data analyses were performed by analysis of variance (ANOVA). One-way ANOVA was applied to determine differences ($p < 0.05$) using SAS, version 8.1.

RESULTS AND DISCUSSION

DNA Gyrase Purification and Supercoiling Assays. The purity and molecular mass of the gyrase subunit in the final protein preparations were evaluated by SDS−PAGE. The purity of each subunit was determined to be higher than 95%, and their apparent molecular mass was consistent with the predicted molecular mass of the polypeptides encoded by gyrA (96.9 kDa) and gyrB (89.9 kDa) (Figure 2). DNA gyrase catalyzes the supercoiling of DNA in an ATP-dependent reaction. The expressed gyrase subunits were stable, and their activities were determined by the DNA supercoiling assay. Enzymatic activities of DNA gyrase reconstituted from their purified subunits were monitored by the introduction of relaxed supercoiled pBR322 plasmid DNA. The specific activity for DNA gyrase was calculated to be 2.7 U/μg, where a unit of enzyme activity is defined as the amount of enzyme needed to supercoil 50% of substrate in 1 h at 37 °C under the assay conditions described above.[22] The recombinant *E. coli* DNA gyrase was found to be enzymatically active, as evidenced by supercoiling relaxed pBR322 DNA (Figure 3).

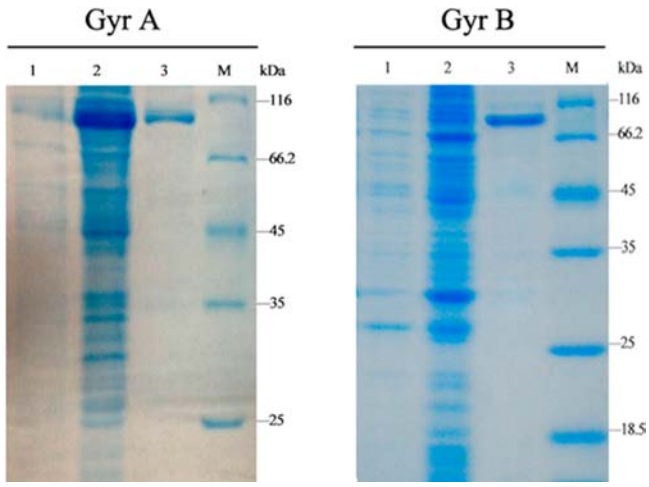

Figure 2. SDS−PAGE analysis of recombinant A and B subunits of *E. coli* DNA gyrase. Gyr A: lane M, protein maker; lane 1, recombinant bacterium (harboring pET-28-GA) induced without IPTG; lane 2, recombinant bacterium (harboring pET-28-GA) induced with IPTG; and lane 3, purified DNA gyrase A protein. Gyr B: lane M, protein maker; lane 1, recombinant bacterium (harboring pGEX-GB) induced without IPTG; lane 2, recombinant bacterium (harboring pGEX-GB) induced with IPTG; and lane 3, purified DNA gyrase B protein.

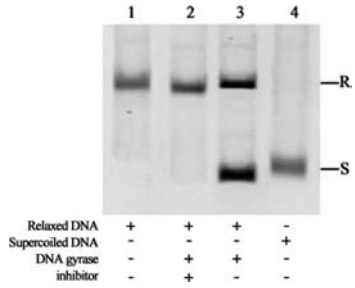

Figure 3. Agarose gel electrophoresis of recombinant *E. coli* DNA gyrase assays. Lane 1, 2, and 3 reactions contained relaxed pBR322 plasmid as the substrate, and the reaction mixture in lane 2 contained 1 μg/mL ciprofloxacin as an inhibitor. The mobility of the relaxed DNA substrate (R) and the supercoiled DNA product (S) are indicated on the right.

Inhibition of DNA Gyrase by Flavonoids. Using the assay conditions established in the preceding experiments, we investigated the inhibitory effect of the flavonoids on DNA gyrase from *E. coli* (Figure 4). All tested flavonoids demonstrated the ability to inhibit *E. coli* DNA gyrase.[23] The IC_{50} values of the flavonoids varied from 0.037 to 1.89 mg/mL (Table 1). Kaempferol showed higher activity than the other flavonoids (IC_{50} = 0.037 mg/mL), which correlates well with previous work by Teffo et al.,[24] while nobiletin exhibited the lowest activity (IC_{50} = 1.89 mg/mL). As indicated in Table 1, tangeritin, nobiletin, and 5,6,7,4′-tetramethoxyflavone, which contain methoxyl groups, showed lower inhibitory ability than other flavonoids with hydroxyl groups.

Anti-*E. coli* Activity of Flavonoids. The results obtained from the antibacterial assay are presented in Table 1. All tested flavonoids showed inhibitory effects against *E. coli*. However, flavonoids containing hydroxyl groups showed higher inhibitory values than the flavonoids with methoxyl groups. Kaempferol, which has hydroxyl group substitutions at C-3, C-5, C-7, and C-4′, showed the highest activity (MIC_{50} = 25 μg/mL), while nobiletin (PMF), which has methoxyl group substitutions at C-5, C-6, C-7, C-8, C-3′, and C-4′, demonstrated the lowest activity (MIC_{50} = 177 μg/mL).

According to Constantinou et al.,[25] quercetin, myricetin, and kaempferol demonstrated inhibitory activity to DNA gyrase resulting from the obligatory C-4 keto group and hydroxyl group substitutions at C-3, C-7, and C-4′. A good correlation was found between the pIC_{50} values and the corresponding pMIC values for the purified DNA gyrase, as shown in Figure 5 (r = 0.9582). The data suggest that DNA gyrase could be a direct target for antibacterial action of flavonoids; therefore, inducing inhibition of DNA gyrase could be an important mechanism of flavonoids as antibacterial agents.

Structure−Activity Relationship. The structure−antibacterial activity relationship of the three PMFs, three flavones, and four flavonols can be established from the results of DNA gyrase inhibition and antibacterial activity reported in Table 1.

Hydroxyl group substitution is an important aspect. For flavonols, 4′-OH increased antibacterial activity, while 3′-OH and 5′-OH decreased activity. Kaempferol (MIC_{50} = 25 μg/mL) with a hydroxyl group at C-4′, quercetin (MIC_{50} = 36 μg/mL) with a hydroxyl group at C-3′, and galangin (MIC_{50} = 53 μg/mL) without 4′-OH suggest that 3′-OH decreases activity and indicates the importance of 4′-OH in the B ring. It has been previously reported that 4′-OH in flavonoids was beneficial for inhibition of the influenza virus.[26] The MIC_{50}

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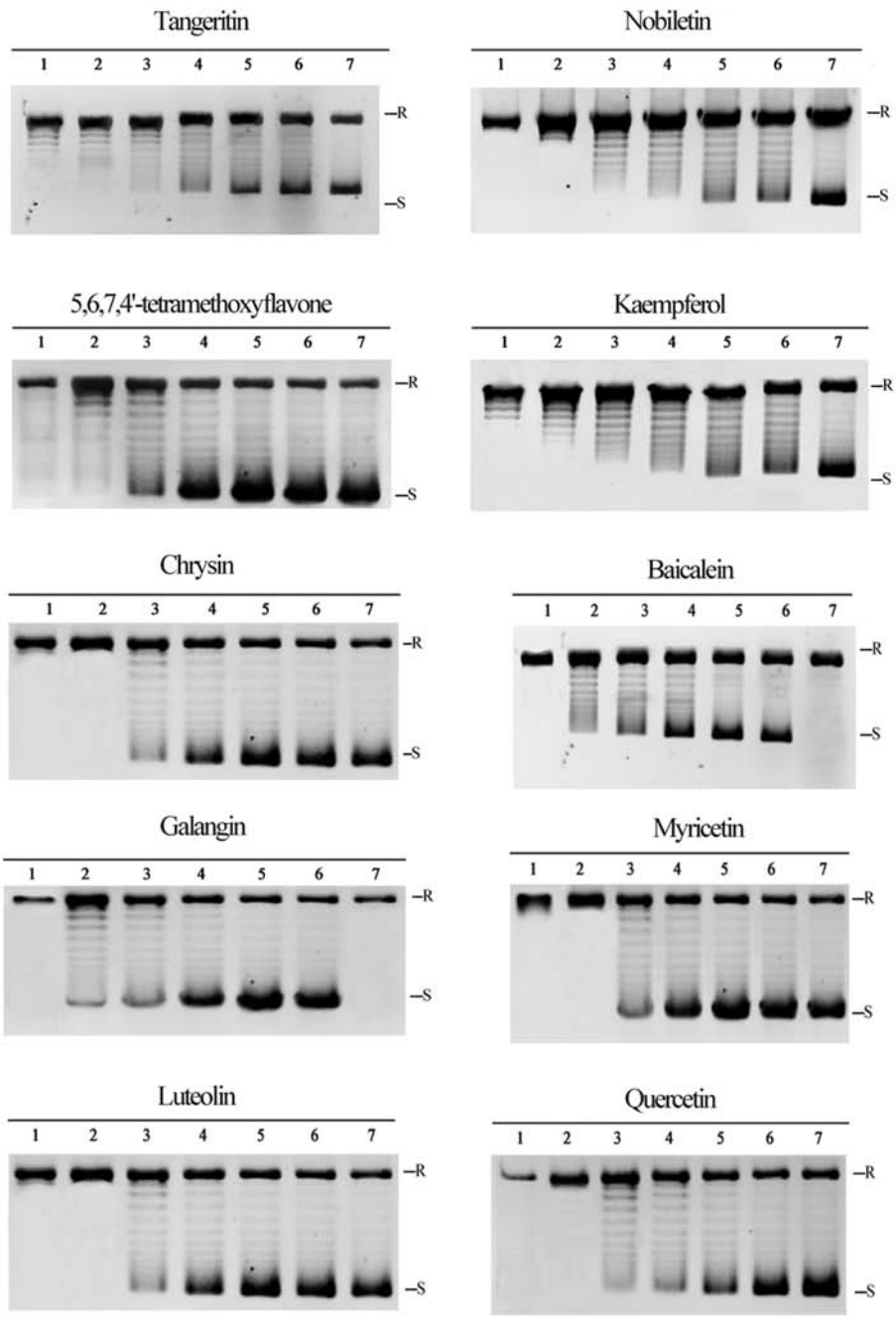

Figure 4. Inhibitory effects of 10 flavonoids on the supercoiling activity of *E. coli* DNA gyrase (R, relaxed pBR322; S, supercoiled pBR322). Tangeritin, 5,6,7,4′-tetramethoxyflavone, nobiletin, chrysin, and kaempferol: lane 1, relaxed pBR322 (R); lane 2, novobiocin (5 μg/mL); and lanes 3−7, 2-fold serial dilution of flavonoids (concentration decreased from left to right). Galangin and baicalein: lane 1, relaxed pBR322 (R); lanes 2−6, 2-fold serial dilution of flavonoids (concentration increased from left to right); and lane 7, novobiocin (5 μg/mL). Quercetin, luteolin, and myricetin: lanes 1−5, 2-fold serial dilution of flavonoids (concentration increased from left to right); lanes 6, novobiocin (5 μg/mL); and lane 7, relaxed pBR322 (R).

values of myricetin (MIC_{50} = 142 μg/mL) and quercetin (MIC_{50} = 36 μg/mL) suggest that 5′-OH also decreases the antibacterial activity of flavonoids.

In comparison to chrysin (MIC_{50} = 37 μg/mL) and luteolin (MIC_{50} = 67 μg/mL), baicalein (MIC_{50} = 71 μg/mL) with 6-OH is less efficient as an antibacterial agent, indicating that the hydroxyl group at C-6 in the A ring could be an adverse factor. Chrysin (MIC_{50} = 37 μg/mL) showed higher antibacterial activity than galangin (MIC_{50} = 53 μg/mL), with their structural difference being galangin having 3-OH in the C ring. The 3-OH in the C ring was considered a prerequisite for antiviral activity of flavonoids,[28] while their antibacterial activity decreased in the presence of 3-OH.[29] The mechanism of action could also account for the difference.

For PMFs, 8-OCH_3 in the A ring appears to be important for antibacterial activity. Tangeritin (MIC_{50} = 137 μg/mL) exhibited stronger inhibitory activity than 5,6,7,4′-tetramethoxyflavone (MIC_{50} = 156 μg/mL), with 8-OCH_3 in the A ring being the only structural difference. This result is in agreement with the research by Liu et al. related to antifungal activities of

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Table 1. IC_{50} (mg/mL) Values of Flavonoids against Purified *E. coli* DNA Gyrase and MIC_{50} (μg/mL) Values of Flavonoids against *E. coli* ATCC 25922[a]

types of flavonoids		IC_{50} (mg/mL)	pIC_{50}[b]	MIC_{50} (μg/mL)	pMIC[c]
PMFs	tangeritin	1.45	2.41	137	3.43
	5,6,7,4′-tetramethoxyflavone	1.74	2.29	156	3.34
	nobiletin	1.89	2.33	177	3.36
flavones	chrysin	0.18	3.15	37	3.84
	baicalein	0.57	2.68	71	3.58
	luteolin	0.51	2.74	67	3.63
flavonols	galangin	0.3	2.96	53	3.7
	quercetin	0.076	3.60	36	3.93
	kaempferol	0.037	3.89	25	4.06
	myricetin	1.18	2.43	142	3.35

[a]All samples were analyzed in triplicate. [b]$pIC_{50} = -\log(IC_{50})$ (mM). [c]$pMIC = -\log(MIC_{50})$ (M).

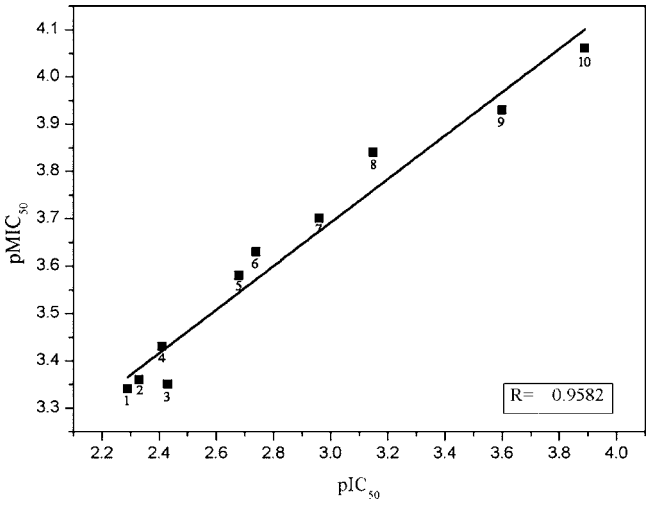

Figure 5. Correlation between the antibacterial activity (MIC_{50} values) of flavonoids and the corresponding concentration of flavonoids inhibiting the supercoiling activity (IC_{50} values) of the DNAgyrase from *E. coli*: 1, 5,6,7,4′-tetramethoxyflavone; 2, nobiletin; 3, myricetin; 4, tangeritin; 5, baicalein; 6, luteolin; 7, galangin; 8, chrysin; 9, quercetin; and 10, kaempferol.

PMFs.[6] Substitution of a lipophilic functional group, such as OCH_3, at C-6 or C-8 has been reported to increase antibacterial activity.[27] Nobiletin (MIC_{50} = 177 μg/mL) has the same OCH_3 groups as tangeritin (MIC_{50} = 137 μg/mL), with the exception of 3′-OCH_3 in the B ring, suggesting that 3′-OCH_3 in the B ring decreases the antibacterial activity.

Tsuchiya et al. indicated that 2′,4′- or 2′,6′-dihydroxylation of the B ring and 5,7-dihydroxylation of the A ring were important for anti-methicillin-resistant *S. aureus* (MRSA) activity.[30] Our results with seven flavonoids having a hydroxyl group at C-5 in the A ring are in agreement for antibacterial activities. The 2′-OH in the A ring (corresponding to position 5 of flavones) of chalcones was shown to be important for antibacterial activity, while 2′-OCH_3 decreases antibacterial activity.[31] Recently, PMFs with hydroxyl groups at C-5 in the A ring have gained attention, because evidence suggests that they have stronger health-promoting biological activities than permethoxylated PMFs because of the presence of these hydroxyl groups. It was shown that 5-hydroxyflavanones and 5-hydroxyisoflavanones with one, two, or three additional hydroxyl groups at the 7, 2′, and 4′ positions inhibited the growth of *Streptococcus mutans* and *Streptococcus sobrinus*.[32]

5,6,7,4′-Tetramethoxyflavone (MIC_{50} = 156 μg/mL), which has methoxyl groups at C-5, C-6, C-7, and C-4′, displayed less inhibitory ability than baicalein (MIC_{50} = 71 μg/mL), which has hydroxyl group substitutions at C-5, C-6, and C-7. Although flavonoids, such as PMFs, with methoxyl groups are less effective antibacterial agents than those with hydroxyl groups at the same position, they are still considered to have good antibacterial activities among natural compounds.

The structure−activity relationship analysis is important in understanding the mechanisms of flavonoids in antibacterial activities. In our study, inhibition of DNA gyrase was shown to be an important mechanism of flavonoids in antibacterial activities. Plaper et al. reported that quercetin inhibits DNA gyrase through two different mechanisms based on the interaction with the ATP-binding site of gyrase.[33] Our study using related flavonoids also supports this conclusion.

The results presented in this study show that, for good inhibitory effect, the hydroxyl group at C-5 in the A ring and C-4′ in the B ring and the methoxyl group at C-3 and C-8 in the A ring are essential, while the presence of the hydroxyl group at C-6 in the A ring, C-3′ and C-5′ in the B ring, and C-3 in the C ring and the methoxyl group at C-3′ in the B ring greatly reduced inhibition of bacteria. The findings of this study provide important information for the exploitation and use of flavonoids as natural preservatives.

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Notes

The authors declare no competing financial interest.

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