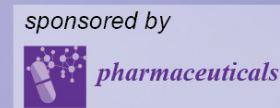




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ANTIBACTERIAL ACTIVITY OF APIGENIN, LUTEOLIN, AND THEIR C-GLUCOSIDES

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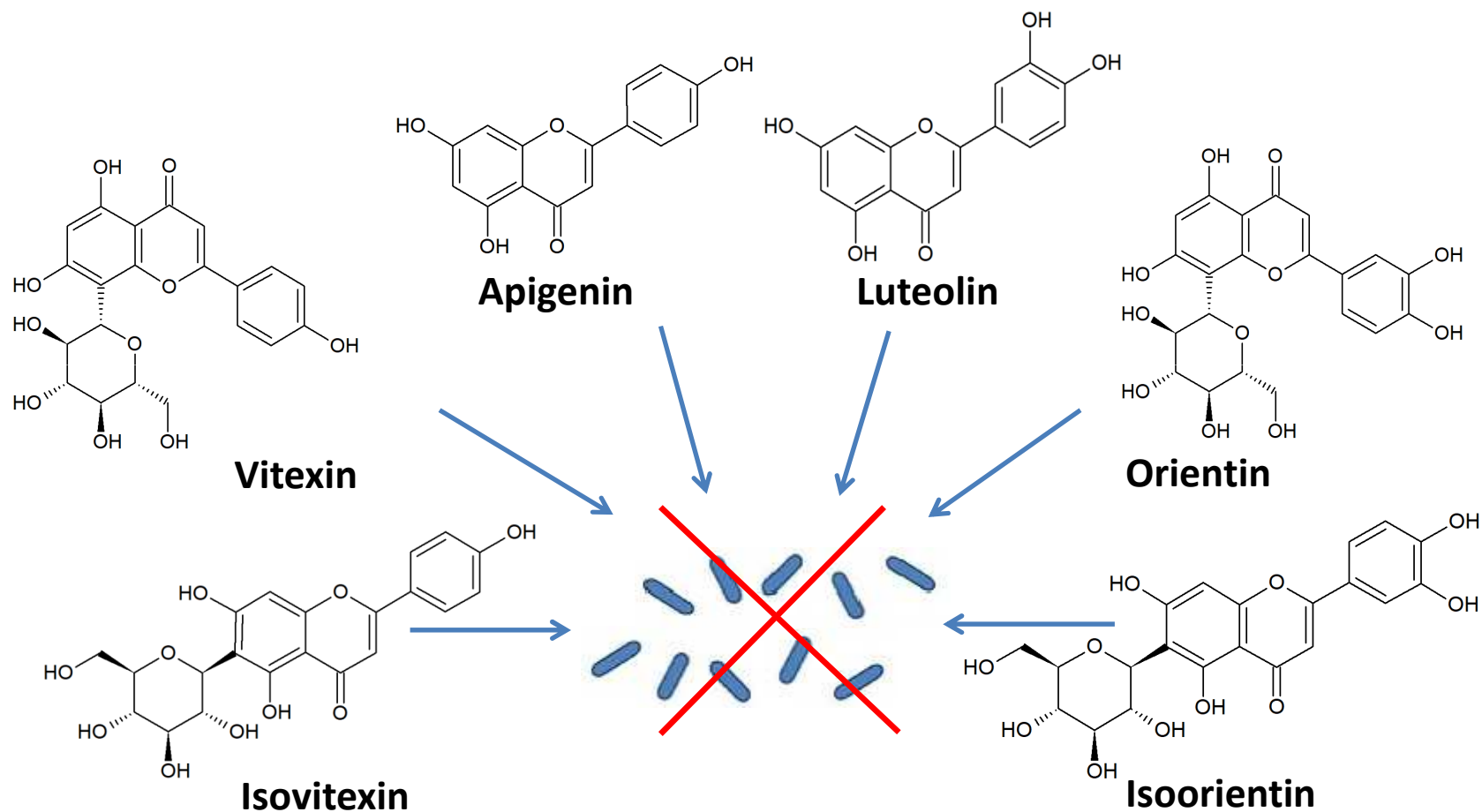
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Graphical Abstract



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Abstract:

Apigenin (4',5,7-trihydroxyflavone) and luteolin (3',4',5,7-tetrahydroxyflavone) are among the most widely distributed flavone aglycones in flowering plants. These metabolites often occur in the form of O- and C-glycosides. In this group, four C-glucosides pay special attention: vitexin (apigenin 8-C-glucoside) and isovitexin (apigenin 6-C-glucoside) as well as orientin (luteolin 8-C-glucoside) and isoorientin (luteolin 6-C-glucoside). The above-mentioned compounds show various biological activities, including antioxidant, anti-inflammatory, cardioprotective, neuroprotective, hepatoprotective, immunomodulatory, and anticancer effects. The aim of the present work was to determine the antibacterial activity of these flavones.

In the *in vitro* tests, there were investigated clinical strains of two Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). The antimicrobial activity of chosen substances was determined by the micro-dilution method according to recommendations of the Clinical and Laboratory Standards Institute (CLSI). Curcumin was used as a positive control.

Our results exhibited a relatively low sensitivity of the tested strains to the plant metabolites. In the case of curcumin – natural compound with known strong antibacterial effect, the minimal inhibitory concentration (MIC) for all species of bacteria was 500 µg/mL. The same level of biological activity was observed for apigenin, luteolin, and their glucosides against *E. coli* and *P. aeruginosa*. Among Gram-positive bacteria, the obtained results showed significant variability. Strains of *S. aureus* demonstrated a weak sensitivity to apigenin (MIC = 500-1000 µg/mL), and were resistant to its derivatives: vitexin and isovitexin (> 1000 µg/mL). Additionally, these compounds poorly inhibited the growth of *E. faecalis* (1000 µg/mL). In turn, luteolin and its C-glucosides (orientin, isoorientin) reached the same values of the MICs: moderate against *S. aureus* (500 µg/mL) and weak for *E. faecalis* (1000 µg/mL).

Our research points to the problem of varied sensitivity and even resistance of some clinical strains of common pathogens to the widespread natural plant compounds, such as flavonoids and other phenolics (e.g., curcumin). It is interesting that apigenin, luteolin, and their C-glucosides were generally more potent against Gram-negative bacteria than Gram-positive ones. A pair of analysed flavone aglycones has a very similar chemical structure, and they did not differ significantly in the antibacterial activity. Similarly, the presence and location of the sugar group in the flavone glucosides usually did not affect the values of the MICs.

Keywords: Aglycones and C-glucosides of flavones, Antibacterial; Minimal inhibitory concentration (MIC)



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Introduction

Apigenin (4',5,7-trihydroxyflavone) and **luteolin** (3',4',5,7-tetrahydroxyflavone) are among the most widely distributed flavone aglycones in flowering plants.

These metabolites often occur in the form of O- and C-glycosides. In this group, four C-glucosides pay special attention: **vitexin** (apigenin 8-C-glucoside) and **isovitexin** (apigenin 6-C-glucoside) as well as **orientin** (luteolin 8-C-glucoside) and **isoorientin** (luteolin 6-C-glucoside).

The above-mentioned compounds show various biological activities, including antioxidant, anti-inflammatory, cardioprotective, neuroprotective, hepatoprotective, immunomodulatory, and anticancer effects.

The aim of the present work was to determine the antibacterial activity of these flavones and their C-glucosides.



Methods

In the study, there were tested two Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). Four strains were tested for each species. None of the chosen strains was multi-resistant.

The minimal inhibitory concentrations (MICs) of apigenin, luteolin, vitexin, isovitexin, orientin, isoorientin were determined by the micro-dilution method described in details in our previous publications [1,2].

Flavonoids were dissolved in 40% water solution of dimethyl sulfoxide (DMSO), which in this concentration has not antimicrobial effect.

[1] Karpiński T.M. Efficacy of octenidine against *Pseudomonas aeruginosa* strains. Eur J Biol Res. 2019, 9, 135-140.

[2] Karpiński T.M., Adamczak A. Fucoxanthin - an antibacterial carotenoid. Antioxidants 2019; 8(8): 239.



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Results

| Plant substance | Tested bacteria | | | |
|-----------------|------------------------------|------------------------------|-------------------------|-------------------------------|
| | <i>Staphylococcus aureus</i> | <i>Enterococcus faecalis</i> | <i>Escherichia coli</i> | <i>Pseudomonas aeruginosa</i> |
| | MIC ($\mu\text{g/ml}$) | | | |
| Apigenin | 500-1000 | 1000 | 500 | 500 |
| Luteolin | 500 | 1000 | 500 | 500 |
| Vitexin | > 1000 | 1000 | 500 | 500 |
| Isovitexin | > 1000 | 1000 | 500 | 500 |
| Orientin | 500 | 1000 | 500 | 500 |
| Isoorientin | 500 | 1000 | 500 | 500 |



Results

Our results exhibited a relatively low sensitivity of the tested strains to the plant metabolites. In the case of curcumin – natural compound with known strong antibacterial effect, the minimal inhibitory concentration (MIC) for all species of bacteria was 500 $\mu\text{g}/\text{mL}$.

The same level of biological activity was observed for apigenin, luteolin, and their glucosides against *E. coli* and *P. aeruginosa*.

Among Gram-positive bacteria, the obtained results showed significant variability.

Strains of *S. aureus* demonstrated a weak sensitivity to apigenin (MIC = 500-1000 $\mu\text{g}/\text{mL}$), and were resistant to its derivatives: vitexin and isovitexin (> 1000 $\mu\text{g}/\text{mL}$). Additionally, these compounds poorly inhibited the growth of *E. faecalis* (1000 $\mu\text{g}/\text{mL}$). In turn, luteolin and its C-glucosides (orientin, isoorientin) reached the same values of the MICs: moderate against *S. aureus* (500 $\mu\text{g}/\text{mL}$) and weak for *E. faecalis* (1000 $\mu\text{g}/\text{mL}$).



Conclusions

Our research points to the problem of varied sensitivity and even resistance of some clinical strains of common pathogens to the widespread natural plant compounds, such as flavonoids and other phenolics (e.g., curcumin).

It is interesting that apigenin, luteolin, and their C-glucosides were generally more potent against Gram-negative bacteria than Gram-positive ones.

A pair of analysed flavone aglycones has a very similar chemical structure, and they did not differ significantly in the antibacterial activity.

Similarly, the presence and location of the sugar group in the flavone glucosides usually did not affect the values of the MICs.

