Flavonoids form a widely distributed class of naturally occurring pigments present in vascular plants, and are responsible for much of the coloring in nature. They occur in fruits, vegetables, nuts, seeds, stems and flowers as well as tea, wine, propolis and honey. Therefore, flavonoids are an integral part of the human diet (1, 2). They have played major roles in successful medical treatments in ancient as well as modern times. Flavonoids exhibit broad spectrum of biological activities: cardiovascular protection (1), antioxidant (3), anticancer (4), anti-inflammatory and antimicrobial activity (5).

The antimicrobial properties of flavonoids were proposed for both development of new food preservation and development of therapies for the treatment of various microbial infections, considering the increase in microbial resistance against antibiotic therapy (6). Nowadays, about 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs. The development of novel antibiotics and/or new generations of phytopharmaceuticals that can reverse the resistance to well-established therapeutic agents that have lost their original effectiveness is of great importance. Several recent reviews and studies have reported the antibacterial efficacy of flavonoids against antibiotic-resistant bacteria (7-9). However, flavonoids application is restricted due to several factors including diversified potency, molecule toxicity, difficult purification strategies or their insolubility in water. Much better solubility is shown by some sulfonic derivatives of flavonoids and, at the same time, they retain the properties of the parent compounds. The sulfonic quercetin derivative NaQSA and the sulfonic morin derivative NaMSA, are characterized by good aqueous solubility and they have been applied in the studies on detoxification of mercury, cadmium and lead in rats (10-12). It is worth emphasizing that NaQSA and NaMSA exert low toxicity (13). Therefore, they are supposed to be useful for a therapy.

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flavonoid derivatives are discussed only in our previous papers (14, 15). However, our studies have focused more on the activity of complexes of metal ions with flavonoids than on activity of the parent compounds. In (14), we presented the antimicrobial action of quercetin-5'-sulfonic acid (QSA) and its complexes with the Zn(II), Fe(II) and Mg(II) ions against S. aureus 209 A, C. albicans 102, E. coli 99-4 and A. niger. Among studied complexes only the complex of Zn(II) has a wide spectrum of activity and exhibits inhibition towards the bacteria and fungi studied under the test conditions. The QSA and its complexes with Zn(II) ions showed lower activity against S. aureus than ampicillin of about 50%. QSA shows no inhibition of growth of the other studied strains.

The antibacterial activity of morin, NaMSA and complexes of La(III), Gd(III) and Lu(III) with morin were also tested (15). The chosen antibiotic sensitive strains E. coli G (-), K. pneumoniae G (-) and S. aureus G (+) were isolated from hospital patients. From the investigation it follows that the most effective inhibitor against E. coli and S. aureus was morin. The same activity against E. coli exhibited the complex of La(III). Both the complex of La(III) and Gd(III) have inhibited the S. aureus growth, MIC = 0.75 µg/mL. Moreover, only the complex of Gd(III) at concentration higher than 0.3 µg/mL showed an inhibitory effect on K. pneumoniae. The MICs for NaMSA against S. aureus and K. pneumoniae were 1.2 and 6.0 µg/mL, respectively, and against E. coli was 60 µg/mL.

The aim of this study is to compare the antibacterial activity of naturally occurring flavonoids: morin, quercetin and chemically synthesized non-natural analogs, like sodium salt of quercetin-5'-sulfonic acid (NaQSA) and sodium salt of morin-5'-sulfonic acid (NaMSA) (Fig. 1). Three commercially available antimicrobial standards Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and S. aureus (ATCC 29213) were used as controls.

Table 1. The MIC (µg/mL) values for flavonoids and their sulfonic derivatives (dilution method).

<table>
<thead>
<tr>
<th>MICROORGANISM</th>
<th>QUERCETIN</th>
<th>MORIN</th>
<th>NaQSA</th>
<th>NaMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ATCC 25922</td>
<td>62.5</td>
<td>3.9</td>
<td>1000.0</td>
<td>62.5</td>
</tr>
<tr>
<td>E. coli clinical isolates</td>
<td>62.5</td>
<td>3.9</td>
<td>62.5</td>
<td>31.2</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>62.5</td>
<td>3.9</td>
<td>31.2</td>
<td>31.2</td>
</tr>
<tr>
<td>P. aeruginosa clinical isolates</td>
<td>62.5</td>
<td>62.5</td>
<td>1000.0</td>
<td>31.2</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>62.5</td>
<td>31.2</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>S. aureus clinical isolates</td>
<td>62.5</td>
<td>31.2</td>
<td>31.2</td>
<td>31.2</td>
</tr>
</tbody>
</table>

Figure 1. Structure of quercetin, morin, rutin, sodium salt of quercetin-5'-sulfonic acid (NaQSA) and sodium salt of morin-5'-sulfonic acid (NaMSA)
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27853), *Staphylococcus aureus* (ATCC 29213) and three antibiotic resistant strains isolated from hospital patients *E. coli* – producing Extended-Spectrum Beta Lactamase (ESBL), *P. aeruginosa* – carbapenem resistant and *S. aureus* – methicillin-resistant (MRSA) were selected to identify the antibacterial spectrum of the compounds using two methods: the dilution method and the cylinder-plate diffusion method. Based on the obtained minimum inhibitory concentration (MIC) values, the structure-activity relationship was speculated to assess a leading compound for the therapy of antibiotic-resistant infections. Taking into account MIC values, compounds with MIC ≥ 100 µg/mL are considered not worth of interest and those with MIC ≤ 100 µg/mL are considered very interesting (16).

**EXPERIMENTAL**

**Reagents**

The flavonoids were obtained as follows: quercetin (C15H10O7) from Riedel de Haën, Germany and morin (C15H10O7) from International Enzymes Ltd., England. All reagents were analytically pure.

NaQSA and NaMSA were synthesized according to the methods described previously (17). Molecular composition of the products was confirmed by elemental analysis of C, H and S, the number of crystalline water molecules was determined by gravimetric and derivatographic method, and sodium content was established by atomic spectrometry. Spectrophotometric characteristics of the NaQSA and NaMSA were found to be concordant with literature data (18).

The aqueous solubility of NaQSA at 22 ± 1°C was estimated as 5.0 × 10−3 mol/L, while the aqueous solubility of NaMSA under the same conditions was 2.7 × 10−2 mol/L.

**Antibacterial test**

The *in vitro* antibacterial activities of flavonoids and their sulfonic derivatives were tested using two methods: the dilution method and the cylinder-plate diffusion method.

The minimal inhibitory concentration (MIC) of the above mentioned strains was determined using the progressive dilution method in liquid medium containing 3.9 to 1000 µg/mL of the compound being tested with the exception of quercetin (19). The activity of quercetin was tested using concentrations: 1000, 125 and 62.5 µg/mL. An appropriate amount of each compound was dissolved in dimethyl sulfoxide (DMSO) to make the desired concentration. The serial dilutions of all compounds were prepared in phosphate buffer (19). Each solution was inoculated with 0.01 mL of one of the 24-h bacterial cultures previously prepared. The inoculated solutions were incubated at 37°C for 24 h. After incubation, the bacterial turbidity was estimated. The results are collected in Table 1. NaQSA showed a low activity and for that reason it was rejected in further investigations.

The cylinder-plate diffusion method (19) was used to investigate antibacterial effects of quercetin, morin and NaMSA. The susceptibility of a bacterium towards flavonoids and their derivatives was tested by measuring the bacteriostatic diameter. The investigation was carried out using two concentrations of rutin, morin and NaMSA: the lower one was nearby the MIC value obtained in dilution method, and the second was ten times higher. In the case of quercetin the concentration were 7.81 and 78.1 µg/mL. Nutrient agar was poured in sterile Petri dishes and allowed to solidify. The bacteria were cultured at 36°C for 24 h in an incubator and added to top agar. Then, the top agar was placed in a plate. Four sterile platinum cylinders of 6 mm diameter

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Quercetin</th>
<th>Morin</th>
<th>NaMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>78.10 µg/mL</td>
<td>7.81 µg/mL</td>
<td>39.10 µg/mL</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>13.4</td>
<td>13.2</td>
<td>15.0</td>
</tr>
<tr>
<td><em>E. coli</em> clinical isolates</td>
<td>15.2</td>
<td>13.2</td>
<td>14.2</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> ATCC 27853</td>
<td>17.2</td>
<td>14.6</td>
<td>18.2</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolates</td>
<td>13.5</td>
<td>11.7</td>
<td>14.4</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 29213</td>
<td>6.0</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td><em>S. aureus</em> clinical isolates</td>
<td>6.0</td>
<td>6.0</td>
<td>-</td>
</tr>
</tbody>
</table>
were put in the agar and completely filled with the test solutions. The plates were then kept at 37°C for 24 h. The width of the growth inhibition zone around the cylinder was measured after incubation (19). Four samples were taken for each treatment. The antibacterial activity of compounds is summarized in Table 2.

RESULTS AND DISCUSSION

Pathogenic strains, of species such as Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus are known as multidrug-resistant (MDR) organisms and are a particular hazard to medical practices. Virtually, no antibiotics are available for their treatment. This makes, that development of new antibacterial drugs an urgent need.

In order to search for new compounds that can be used for the treatment of bacterial infections, this paper presents the in vitro antibacterial activity of naturally occurring flavonoids, namely morin, quercetin and their sulfonic derivatives against three standard bacterial strains: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853) and Staphylococcus aureus (ATCC 29213) as well as three antibiotic resistant strains isolated from hospital patients E. coli – ESBL, P. aeruginosa – carbapenem-resistant and S. aureus – MRSA.

To compare the antibacterial activity of flavonoids and their sulfonic derivatives we first tested them for ability to limit the growth of three standard bacterial strains. Morin inhibited the growth of Gram-negative bacteria strains, E. coli and P. aeruginosa at the lowest tested concentration, MIC = 3.9 µg/mL. In the case of S. aureus, the antimicrobial activity of morin was lower and MIC was equal to 31.2 µg/mL. Quercetin exhibited inhibition towards the standards at the concentration – 62.5 µg/mL. Among the two sulfonic derivatives of flavonoids, the derivative of morin (NaMSA) exhibited stronger activity against all standards. However, both NaMSA and NaQSA had a similar activity against S. aureus, MIC = 3.9 µg/mL.

To further explore their antibacterial activity, all the compounds investigated were assayed in vitro against isolated drug-resistant strains. As seen in Table 1, the tested compounds displayed significant antibacterial activity against all isolated strains. As before, morin showed high activity. The efficiency of morin was raised in the series: P. aeruginosa < S. aureus MRSA < E. coli ESBL. The MIC values ranged from 3.9 to 62.5 µg/mL. Good activity against all clinical isolates was observed by NaMSA (MIC 31.2 µg/mL). On the other hand, NaQSA was active against MRSA and EBSL with MIC values of 31.2 and 62.5 µg/mL, respectively. Importantly, the MIC values for the compounds approach those of traditional antibiotics.

The susceptibility of certain strains of bacteria towards test compounds was also judged by measuring the inhibition diameter. The results are summarized in Table 2. From the above investigations it follows that the increase of polyhydroxyflavones concentration has not any meaningful influence on their antibacterial activity. It is probably the result of poor and slow diffusion of these compounds in agar. It was stated that the largest inhibition diameters occur in the case of morin (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) and NaMSA (Staphylococcus aureus ATCC 29213 and Pseudomonas aeruginosa clinical isolates).

The structure of drugs is closely related to their biological properties. Thus, the antibacterial activity of flavonoids is determined by their structural features. The flavonoids tested in this study belong to polyhydroxyflavones. They have C-4 keto group and hydroxyl group substitutions at C-3, C-5 and C-7 and have two hydroxyl groups in the ring B, quercetin 3′,4′ and morin 2′,4′. Since quercetin differs from morin in the positioning of just one hydroxyl group but is less antibacterial, this would suggest that the position of the free hydroxyl groups in the molecule are important for its activity (20). Tsuchiya et al. (21) suggested that 2′,4′-dihydroxylation of the B ring and 5,7-dihydroxylation of the A ring in the flavone structure are important for significant antistaphylococcal activity. The structure of the A ring may be of less importance for antibacterial activity than of the ring B. A more recent paper (22) also reports the importance of a hydroxyl group at position 2′ of flavonoids for antibacterial activity. This is possibly the reason why quercetin (2′ unsubstituted) exhibited weaker activity than morin against tested strains.

In order to improve the properties of flavonoids, an effort was made to synthesize their new derivatives. Chemical modifications were made to the B-ring, a position that has been implicated in its biological activity. Substitution of hydrophilic sulfonic group in position 5′ greatly increases the aqueous solubility of the compounds. Since the more hydrophilic compounds show higher antibacterial activity than the less hydrophilic, it was expected that the sulfonic derivatives may be better inhibitors of bacterial growth than the parent compounds. However, such result for both derivatives NaMSA and NaQSA was obtained only for S. aureus (standard – 3.9 µg/mL and MRSA – 31.2
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µg/mL). For the other strains, NaMSA showed less efficacity than morin. NaQSA had higher activity than quercetin against P. aeruginosa ATCC 27853 but marginal effects against E. coli ATCC 25922 and P. aeruginosa – carbapenem-resistant. These observations indicate that substitution of the sulfo group at position 5′ on the lateral phenyl ring enhances only antistaphylococcal activity of the flavonoids. Comparing to our previous reports (14, 15), in the present study the same compounds showed slightly different activity. These inconsistencies may be due to a difference in strains used.

CONCLUSION

The results presented in this paper provide evidence that naturally occurring flavonoids morin and quercetin and their sulfonic derivatives NaMSA and NaQSA exhibit antibacterial activity. Among polyhydroxyflavones used in this investigation, morin exhibits the highest antibacterial activity against tested strains. The sulfonic derivatives NaQSA and NaMSA are potent antimicrobial inhibitors against S. aureus strain in the range of MIC = 3.9 to 31.2 µg/mL. Although the MIC values displayed by NaMSA and NaQSA are relatively high compared with useful antibiotics, the sulfonic derivatives have the potential to be developed into antibiotics because of their non-toxic nature.

REFERENCES


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