ANTIBACTERIAL, ANTIFUNGAL AND ANTIMYCOBACTERIAL STUDIES ON SOME SYNTHETIC DIMETHOXY FLAVONES

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ABSTRACT

Development of microbial resistance to various existing antimicrobial agents has become a serious public health concern and the search for new classes of antimicrobial agents is a challenging task. The focus is on naturally occurring substances and their derivatives. Flavonoids are a group of naturally occurring agents and have been shown to possess good antimicrobial activity. The present study examines the antibacterial, antifungal and antimycobacterial activities of three synthetic dimethoxy flavones, 3,6-dimethoxy flavone, 6,2’-dimethoxy flavone and 6,3’-dimethoxy flavone. All the three compounds showed a similar pattern of antibacterial activity. They were most active against E. coli, K. pneumoniae, S. typhi, S. paratyphi B, C. albicans and M. tuberculosi and were able to inhibit their growth even at concentrations of 160 μg/ml. E. faecalis and M. crococi were inhibited at concentration of 240 μg/ml whereas S. aureus could be inhibited at concentration of 320 μg/ml only. All the three compounds showed excellent antifungal activity against all the fungi tested and were able to inhibit their growth at the concentration of 5 μg/ml. The three compounds showed different activity pattern against M. tuberculosi. While 3,6-dimethoxy flavone and 6,2’-dimethoxy flavone showed MIC values of greater than 100 μg/ml, 6,3’-dimethoxy flavone could inhibit the growth of the organism at 100 μg/ml.

Keywords: Flavonoids, dimethoxy flavones, MIC, zone of inhibition, REMA.

INTRODUCTION

Antimicrobial resistance has become a serious public health concern with serious economic and social ramifications. The changing resistance pattern of various microorganisms to existing antimicrobial agents continues to be a constant threat for both developed and developing countries. Resistance to some agents can be overcome by modifying the dosage regimes such as high dose therapy or by inhibiting the resistance mechanism such as the use of beta-lactamase enzyme inhibitors. Other mechanisms of resistance can be overcome by using totally different class of compounds. Structural modification of existing class of antimicrobials to which resistance has developed has proven to be a useful method of extending the life span of antifungal agents like the azoles.

Natural products have been shown to be a potential source of anti-infective agents-the classic example being that of Penicillin and Tetracycline. Flavonoids are a class of natural compounds possessing a wide range of pharmacological activities. They are found in fruits, vegetables, nuts, seeds, stems and flowers as well as in beverages such as wine and tea. They form a common part of the human diet. The role of flavonoids in the plants was thought to be to promote pollination. The other roles attributed to them were to promote physiological survival of the plant by protecting them from fungal pathogens and UV-B radiation. They are also known to be involved in photosensitization, energy transfer, actions of plant growth hormones and regulators, control of respiration and photosynthesis, morphogenesis etc.

At present the flavonoids are the subject of medical research and there are several reports of their anti-inflammatory activity, oestrogenic activity anti-allergic activity, anti-oxidant activity, antitumour activity and antimicrobial activity. Historically preparations containing flavonoids as the principal active constituent have been used to treat various human disorders. The Old Testament refers to the healing property of Propolis. This antimicrobial property has been attributed to the presence of high proportions of flavonoids. The activity of flavonoids is probably due to their ability to form complexes with extracellular and soluble proteins and with bacterial cell walls.

The antimicrobial activity of flavonoids and flavonoid rich fractions of several plant extracts have been reported. The antifungal properties of various naturally occurring flavonoids have also been reported. Flavonoids such as 2’-methoxy 4’, 5’ methylene dioxyfuran flavone, 3, 5 dimethoxy flavone and 5, 7, 3’-trihydroxy flavone and extracts of Artocarpus altilis, Limnophila geoffrayi have been shown to possess antibacterial properties. Since most of the scientific reports are on the natural flavonoids, the present work was undertaken to determine whether the synthetic flavonoids also possess antibacterial and antimycobacterial properties.

MATERIALS AND METHODS

The dimethoxy flavones used in the study were the synthesized flavone (3,6-DMF), 6,2’-dimethoxy flavone (6,2’-DMF) and 6,3’-dimethoxy flavone (6,3’-DMF) were synthesized using standard procedures at Research Organics, Chennai-41, Tamilnadu, India. The melting point, UV spectra and IR spectra of the synthesized compounds were compared with standard samples.

The various organisms used in the present study include Staphylococcus aureus, Escherichia coli, Micrococcus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi B, Citrobacter freundii and Serratia marcescens. The fungi used for the study were Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus and Candida albicans. Mycobacterium tuberculosis H37Rv strain was used for the antitymocobacterial study.

Mueller Hinton agar (MH) medium was used for the growth of the bacteria, Sabourad’s Dextrose agar medium was used for the growth of fungi and M. tuberculosis was grown in Middlebrook pH9 broth supplemented with 10% OADC and 0.5% glycerol.

The three test compounds were dissolved in dimethyl formamide and these solutions in different concentrations were used for the antimicrobial activity.

ANTIBACTERIAL ACTIVITY

MIC determination

The test samples were introduced aseptically into sterilized Petri dishes and mixed with MH agar medium to get final concentrations ranging from 40-400 μg/ml and was allowed to set. The plates with different concentrations of test samples were inoculated with a loopful of the culture at the labeled spots. The plates were incubated at 37°C for 24h. The results were read by the presence or absence of growth of the organisms. The minimum concentration with no...
growth was noted as minimum inhibitory concentration (MIC) (Table 1).

Antibiotic disc diffusion technique 20

The pathogenic strains were then seeded on the MH agar media in a Petri dish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry and then the Ciprofloxacin 5µg test drug and dimethyl formamide discs were placed on the seeded medium plates and maintained at 4°C for 30min to allow perfusion of drugs being tested. The plates were then incubated at 37°C for 24h. The zone of inhibition was then measured (Table 2).

Antifungal activity 21

The test samples (1ml) were introduced aseptically into sterilized Petri dish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The plates were incubated at 37°C for 24h. The zone of inhibition was then measured (Table 2).

Antimycobacterial activity

The antimycobacterial activity of the compounds was determined by the resazurin microplate assay (REMA). The solutions of test compounds, in concentrations of 1, 10 and 100 µg/ml were added to fresh medium in the wells of 96-well microplates to which 50µl of the inoculum was added. Rifampicin (1µg/ml) served as the positive control. Negative control wells contained the solvent alone. The plates were incubated at 37°C for 24h. Blue in colour wells containing test compound would indicate inhibition of growth and pink colour indicates lack of inhibition of growth. The MIC values are given in Table 4.

RESULTS

Growth of all cultures was seen with the solvent control and in the positive culture plate. All the three test compounds showed a similar pattern of activity. All of them at a concentration of 160µg/ml inhibited the growth of E. coli, K. pneumoniae, P. aeruginosa, S. typhi, S. paratyphi B, Citrobacter and S. marcescens. The growth of E. faecalis and Micrococcus was inhibited at the concentration of 240µg/ml whereas S. aureus could be inhibited only at a concentration of 320µg/ml.

All the compounds showed inhibitory effect on all the pathogenic bacteria with varying degrees of zone of inhibition. The maximum zone of inhibition was seen with the standard drug Ciprofloxacin at a concentration of 5µg. The three dimethoxy flavones tested showed activities less than Ciprofloxacin as seen with decreased zone of inhibition values. Maximum zone of inhibition values were seen with 3,6-DMF against S. aureus, E. faecalis, K. pneumoniae, P. aeruginosa, S. typhi and S. marcescens. 6, 2’-DMF showed maximum activity against Micrococcus, E. coli and C. freundii. The only organism against which 6, 3’-DMF showed maximum activity was S. paratyphi B.

All the three methoxy flavones showed an exactly similar pattern of antifungal activity. They could not inhibit the growth of all the five fungi at the concentration of 1.25 and 2.5µg/ml. They could not completely inhibit the growth of all fungi at the concentration of 5 µg/ml and above.

Rifampicin was capable of inhibiting the growth of Mycobacterium tuberculosis H37Rv at the concentration of 10µg/ml. Both 3, 6-DMF and 6, 2’-DMF were not able to inhibit the growth of the micro-organism even at 100µg/ml whereas 6, 3’-DMF inhibited the growth of the micro-organism at 100µg/ml.

Table 1: Anti-bacterial activity of the dimethoxy flavones.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test compound</th>
<th>MIC values in µg/ml</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>3,6-DMF</td>
<td>&gt;240</td>
</tr>
<tr>
<td>2</td>
<td>6,2’-DMF</td>
<td>&gt;240</td>
</tr>
<tr>
<td>3</td>
<td>6,3’-DMF</td>
<td>&gt;240</td>
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Table 2: Antimycobacterial activity of the dimethoxy flavones.

<table>
<thead>
<tr>
<th>Zone of inhibition(mm)</th>
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<tr>
<td>Ciprofloxacin 5µg</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td>Micrococcus</td>
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<tr>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Salmonella typhi</td>
</tr>
<tr>
<td>Salmonella paratyphi B</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
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<tr>
<td>Serratia marcescens</td>
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Table 3: Anti-fungal activities of the dimethoxy flavones.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test compound</th>
<th>MIC (µg/ml)</th>
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<tbody>
<tr>
<td></td>
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<td>1</td>
</tr>
<tr>
<td>1</td>
<td>3,6-DMF</td>
<td>&gt;2.5&lt;5</td>
</tr>
<tr>
<td>2</td>
<td>6,2’-DMF</td>
<td>&gt;2.5&lt;5</td>
</tr>
<tr>
<td>3</td>
<td>6,3’-DMF</td>
<td>&gt;2.5&lt;5</td>
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The reason for the lesser material activity of methanolic extract of Psidium guajava leaves is on the increase of various gram positive and gram negative bacteria. They were able to inhibit the growth of the gram negative bacteria at a concentration of 160µg, whereas the gram positive bacteria Enterococci, Micrococc and S. aureus could only be inhibited at higher concentrations of 240µg and 320µg/ml.

It has been reported that activity against gram positive bacteria such as S. aureus is shown by flavonoids with a hydroxy group in the ring B. The absence of this group could be the reason for the lesser degree of antibacterial activity of flavonoids extracted from leaves of Psidium guajava.

**DISCUSSION**

The flavonoids are increasingly becoming the subject of anti-infective research. Several flavonoids have been known to possess antibacterial, antifungal and antiviral activities. In the present study, all the three compounds behaved similarly against the various gram positive and gram negative bacteria. It was observed that activity against gram positive bacteria such as S. aureus is shown by flavonoids with a hydroxy group in the ring B. The absence of this group could be the reason for the lesser degree of antibacterial activity of flavonoids extracted from leaves of Psidium guajava.

Owing to the ability of flavonoids to inhibit spore germination of plant pathogens, it has been proposed that they might be useful against pathogenic fungi in man. The present study, the test compounds showed excellent antifungal activities against all the pathogenic fungi tested. Similar to the antibacterial activity, in this case too, all the three compounds exhibited a similar pattern of activity. The fact that they are active against Aspergillus flavus could indicate that they may be of use in immunocompromised patients as this fungus has been shown to cause invasive disease in such patients.

The discovery of new antitubercular agents is also essential as the incidence of drug resistant tuberculosis is on the increase worldwide. A number of flavonoids have shown certain amount of antitubercular activity. In the present study it was seen that only 6,3'-DMF at the highest concentration of 100µg/ml inhibited the growth of Mycobacterium tuberculosis while the other two compounds did not inhibit the growth at this concentration. Rifampicin, the standard drug, could inhibit the growth at a concentration of 10µg/ml. Hence, it may be assumed that the methoxy group at 3' position conferred a higher degree of antitubercular activity.

**CONCLUSION**

This study has shown that the synthetic dimethoxy flavones 3, 6-dimethoxy flavone, 6, 2'-dimethoxy flavone and 6, 3'-dimethoxy flavone possess very good antifungal effect and good antibacterial effect. Of the three compounds, 6, 3'-dimethoxy flavone also showed good antitubercular activity. Hence these compounds may have a potential to be used as effective antibacterial and antifungal agents.

**REFERENCES**