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Research Article

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.pnemoniae, S.typhi, S.paratyphi B, Citrobacter and S.marscesens* and were able to inhibit their growth even at concentrations of 160µg/ml. E.faecalis and Micrococci were inhibited at concentration of 240µg/ml whereas *S.aureus* could be inhibited at concentration of 320µg/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 5µg/ml.The three compounds showed different activity pattern against *M.tuberculosis.* 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 100µg/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 regimens 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 [1]. 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 [2].

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 [3]. 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[2].

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[2]. Historically preparations containing flavonoids as the principal active constituent have been used to treat various human disorders [3]. The Old Testament refers to the healing property of Propolis. This antimicrobial property has been attributed to the presence of high proportions of flavonoids [4, 5]. 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 [2,6,7,8,9,10]. The antifungal properties of various naturally occurring flavonoids have also been reported [11, 12,13,14,15]. Flavonoids such as 2'-methoxy 4', 5' methylene dioxyfurano flavone, 3, 5 dimethoxy flavone and 5, 7, 3' –trihydroxy flavone and extracts of *Artocarpus altilis, Limnophila geoffrayi* have been shown to possess antitubercular properties [16, 17, 18].

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 3,6-dimethoxy 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, Micrococci, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi B, Citrobacter freundii and Serratia marsescens.* 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 antimycobacterial 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 study.

ANTIBACTERIAL ACTIVITY

MIC determination [19]

The test samples were introduced aseptically into sterilized Petri dishes and mixed with MH agar medium to get final concentrations ranging from 40-400µg/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 tubes to get final concentrations of 1.25-160µg/ml. The tubes with different concentrations of test samples were inoculated with the fungal strains. The tubes were then incubated at 37°C for 7 days. The results were read by the presence or absence of growth of the organisms and the MIC determined (Table 3).

Antimycobacterial activity

The antimycobacterial activity of the compounds was determined by the resazurin microplate assay (REMA) [22]. 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. The growth control wells contained medium and the inoculum. Rifampicin (1µg/ml) served as the positive control. Negative control wells contained the solvent alone. The plates were incubated at 37°C for 24h for seven days. After incubation, 15µl of 0.01% resazurin in sterile water was added and incubated at 37°C for 24h. Blue colour in 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. marscesens*. The growth of *E. faecalis* and *Micrococci* 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.marscesens*. 6, 2'-DMF showed maximum activity against *Micrococci*, *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 tuberuculosis* 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.

S.No	Test compound	MIC values in µg/ml									
		1	2	3	4	5	6	7	8	9	10
1	3,6-DMF	>240	>160	>160	>80	>80	>80	>80	>80	>80	>80
		<320	<240	<240	<160	<160	<160	<160	<160	<160	<160
2	6,2'-DMF	>240	>160	>160	>80	>80	>80	>80	>80	>80	>80
		<320	<240	<240	<160	60	<160	<160	<160	<160	<160
3	6,3'-DMF	>240	>160	>160	>80	>80	>80	>80	>80	>80	>80
		<320	<240	<240	<160	<160	<160	<160	<160	<160	<160

1. *Staphyllococcus aureus* 2. *Enterococcus faecalis* 3. *Micrococci* 4. *Escherichia coli* 5. *Klebsiella pneumonia* 6. *Pseudomonas aeruginosa* 7. *Salmonella typhus* 8. *Salmonella paratyphi B* 9. *Citrobacter freundii* 10. *Serratia marscesens*

Table 2: Antibiotic disc diffusion assay of the DMF against various micro-organisms.

S. No	Organism	Zone of inhibition(mm)			
		Ciprofloxacin 5µg	3,6-DMF 320µg	6,2'-DMF 320µg	6,3'-DMF 320µg
1	*Staphyllococcus aureus*	30	18	15	17
2	*Enterococcus faecalis*	30	20	11	18
3	*Micrococci*	35	25	29	20
4	*Escherichia coli*	40	22	25	18
5	*Klebsiella pneumoniae*	40	24	20	22
6	*Pseudomonas aeruginosa*	45	18	15	15
7	*Salmonella typhi*	28	20	18	15
8	*Salmonella paratyphi B*	40	20	15	21
9	*Citrobacter freundii*	40	20	23	22
10	*Serratia marscesens*	30	25	18	21

Table 3: Anti-fungal activities of the dimethoxy flavones

S.No	Test compound	MIC (µg/ml)				
		1	2	3	4	5
1.	3,6-DMF	>2.5<5	>2.5<5	>2.5<5	>2.5<5	>2.5<5
2.	6,2'-DMF	>2.5<5	>2.5<5	>2.5<5	>2.5<5	>2.5<5
3.	6,3'-DMF	>2.5<5	>2.5<5	>2.5<5	>2.5<5	>2.5<5

1. *Aspergillus flavus* 2.*Aspergillus fumigatus* 3.*Rhizopus* 4.Aspergillus *niger* 5.*Candida albicans*

Table 4: Antitubercular activity of the dimethoxy flavones (REMA Assay).

S.No	Test compound	MIC (µg/ml)
1	Rifampicin	>1 <10
2	3,6-DMF	>100
3	6,2'-DMF	>100
4	6,3'-DMF	>10 <100

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. They were able to inhibit the growth of the gram negative bacteria at a concentration of 160µg whereas the gram positive bacteria *Enterococci, Micrococci* 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 hydroxyl group in the ring B [22]. The absence of this group could be the reason for the lesser activity of the test compounds.

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 [23]. In 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 antimycobacterial 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

1. Sharma R, Sharma CL, Kapoor B. Antibacterial resistance: Current problems and possible solutions. Ind J Med Sci 2005; 59(3): 120-129.
2. Cushnie TTP,.Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicr Agents 1995; 26(5): 343-356.
3. Havsteen B. Flavonoids: A class of natural products of high pharmacological potency. Biochem Pharmacol 1983; 32: 1141-1148.
4. Grange JM, Davey RW. Antibacterial properties of Propolis (Bee glue).J R Soc Med 1990; 83:159-160.
5. Bosio K, Avanzini C, D'Avolio A, Ozino O, Savoia D. *In vitro* activity of propolis against *Streptococcus pyogenes*. Lett Appl Microbiol 2000; 31:174- 177.
6. Rattanachaikunsopon P, Phumkkachorn P; Contents and antibacterial activity of flavonoid extracted from leaves of *Psidium guajava.* J Med Plant Res 2010; 4(5): 393-396.
7. Mishra US, Chakraborthy P, Dasgupta A, Dastidar SG *et al*. Potential bactericidal action of a flavonoid fraction isolated from the stem bark of *Butea frondosa*.In vivo 2009; 23(1), 29-32.
8. P.S.Pavithra, N.Sreevidya, R.S.Verma; Antibacterial and antioxidant activity of methanolic extract of *Evolvulus nummularius*. Indian J Pharmacol, 41(5), 233-236 (2009).
9. S.Akroum, D.Bendjeddou, D.Satta, K.Lalaoui; Antibacterial activity and acute toxicity effects of flavonoids extracted from *Mentha longifolia*.Am Eurasian J Sci Res, 4(2), 93-96 (2009).
10. B.S.Jayashree, N.F.Anjum, Y.Nayak, D.Vijaykumar; Synthesis of substituted 3-hydroxy flavones for antioxidant and antimicrobial activity. Pharmacol online, 3, 586-595 (2009).
11. R.N.Prasad, C.Anandi, S.Balasubramaniam, K.V.Pugalendi; Antidermatophytic activity of extracts from *Psoralea corylifolia* (Fabaceae) correlated with the presence of a flavonoid compound.J Ethnopharmacol, 91(1), 21-24 (2004).
12. R.Valsaraj, S.Puspangadan, U.W.Smith *et al*; New anti HIV-1, antimalarial and antifingal compounds from *Terminalia bellarica* J Nat Prod, 60, 739-742 (1997).
13. W.F.Zheng, R.X.Tan , L.Yang , Z.I.Liu ZI; Two flavones from *Artemisia giraldi* and their antimicrobial property. Planta Medica, 62, 160-162 (1996).
14. K.Uddin , A.Sayeed , A.Islam , A.A.Rahman *et al*; Biological activities of extracts and two flavones from *Oroxylum indicum* Vent (Biogoniaceae). Online J Biol Sci, 3(3), 371-375(2003).
15. A.J.Afloyan, J.J.M.Meyer; The antimicrobial activity of 3,5,7-trihydroxy flavones isolatedbfrom the shoots of *Helichrysum aureonitens*. J Ethnopharmacol, 57, 177-178(1997).
16. A.S.Negi, J.K.Kumar, S.Luqman, D.Saikia *et al*; Antitubercular potential of plants: A brief account of some important molecules. Medicinal Res Rev, 30(4), 603-45 (2010).
17. S.Boophong, A.Baramese, P.Kittakoop, P.Puangsombhat; Antitubercular and antiplasmodial prenylated flavones from the root of *Artocarpus altilis.* Chiang Mai J Sci,, 34(3), 339-344 (2007).
18. S.Suksamrarn, O.Poomsing, N.Aroonrerk, T.Punjanon T *et al*; Antimycobacterial and antioxidant flavones from *Limnophila geoffrayi.* Arch Pharm Res, 26(10), 816-820 (2003).
19. J.G.Collee, J.P.Dugauid, A.G.Fraser, B.C.Marimon, M.Mc Cartney; Practical Medical Microbiology, 13th ed. p.161-180 (1989).
20. K.C.Agarwal; Antibiotic sensitivity test by the disc diffusion method - standardization and interpretation. Indian J Path Bact;17(3):149 (1974).
21. M.Finegold, E.J.O.Barron; Bailey, Scott's Diagnostic Microbiology-methods for testing antimicrobial effectiveness, Sydney (1993).
22. A.Martin, M.Camacho, F.Portaels, J.C.Palomino; Resazurin microtitre assay plate testing of *Mycobacterium tuberculosus* susceptibilities to second line drugs: rapid, simple and inexpensive method.Antimicrob Agents Chemother, 47(11), 3616-3619 (2003).
23. A.Mori, C.Nishino, N.Enoki, S.Tawata; Antibacterial activity and mode of plant flavonoids against *Proteus aureus* and *Staphylococcus aureus.* Phytochemistry, 26, 223-224 (1987).
24. J.B.Harborne, C.A.William; Advances in flavonoid research since 1992. Phytochemistry; 55, 481-504 (2000).