



Comparative Antimicrobial Evaluation of Substituted Flavones

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Received: 15-05-2017; Revised: 28-06-2017; Accepted: 15-07-2017.

ABSTRACT

The development of antimicrobial resistance has become a major source of concern for both developed and developing countries. The anti-microbial resistance can be overcome by using new classes of compounds or by structural modification of the existing class of antimicrobials. Natural products are potential sources of anti-infective agents. One such class of natural products are flavonoids, which are polyphenolic compounds widely distributed in the plant kingdom. The present study was undertaken to determine whether synthetic flavonoids also possess antibacterial and antifungal activity. The flavonoids used in the study are 6, 3', 4'-trihydroxy flavone and 3-hydroxy-6, 3'-dimethoxy flavone. The organisms used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Streptococcus mutans* and *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. Of the two compounds, 6, 3', 4'-trihydroxy flavone showed comparatively better antibacterial and antifungal effect.

Keywords: flavones, antibacterial, antifungal, zone of inhibition.

INTRODUCTION

The development of antimicrobial resistance has become a major source of concern for both developed and developing countries.¹ The antimicrobial resistance can be overcome by using new classes of compounds or by structural modification of the existing class of antimicrobials.² The chemical modification of antimicrobial drugs that developed resistance, proved to be effective in extending the activity of antifungal agents such as azoles,³ antiviral agents such as non-nucleoside reverse transcriptase inhibitors,⁴ and various anti-bacterial agents such as β -lactams and quinolones.⁵

Natural products are potential sources of anti-infective agents. One such class of natural products are flavonoids, which are polyphenolic compounds widely distributed in the plant kingdom.⁶ Various experimental systems revealed that flavonoids possess anti-inflammatory, antioxidant, anti-microbial, anti-viral and anti-carcinogenic properties.⁷ Antimicrobial activity has been recognized in naturally occurring flavanoids. These flavonoids possessing antifungal, antiviral and antibacterial activity have been isolated and identified. Quercetin and naringenin have been identified as inhibitors of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus epidermis* etc.⁸ Nobiletin and tangeritin seen in orange peels exhibited fungistatic action towards *Deuterophoma tracheiphila*.⁹

Since most of the scientific reports available are on natural flavonoids, the present study was undertaken to determine whether synthetic flavonoids also possess antibacterial and antifungal activity.

MATERIALS AND METHODS

The flavonoids used in the study 6,3',4'-trihydroxy flavone(6,3',4'-THF) and 3-hydroxy-6,3'-dimethoxy flavone(3-hydroxy-6,3'-DMF) were synthesized using standard procedures at Research Organics, Chennai. The melting point, UV spectra, IR spectra, NMR spectra and LC-MS data of the synthesized compounds were compared with standard samples and authenticated.

The various organisms used in the present antibacterial study include *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Streptococcus mutans* and *Staphylococcus aureus*. The fungi used for the study include *Aspergillus niger* and *Candida albicans*. Muller Hinton Agar Medium was used to study the growth of bacteria while Potato Dextrose agar plates were used for antifungal study.

Streptomycin and clotrimazole were taken as the standards for antibacterial and antifungal activity study respectively.

The two test compounds were dissolved in dimethyl sulphoxide and different concentrations of these solutions were used for the study.

Antibacterial activity

The antibacterial activity was evaluated by agar well diffusion method. Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Streptococcus mutans* and *Staphylococcus aureus* (growth of culture adjusted according to Mc Fards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and



sample of 25, 50, and 100 µg concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.¹⁰ Streptomycin was used as a positive control.

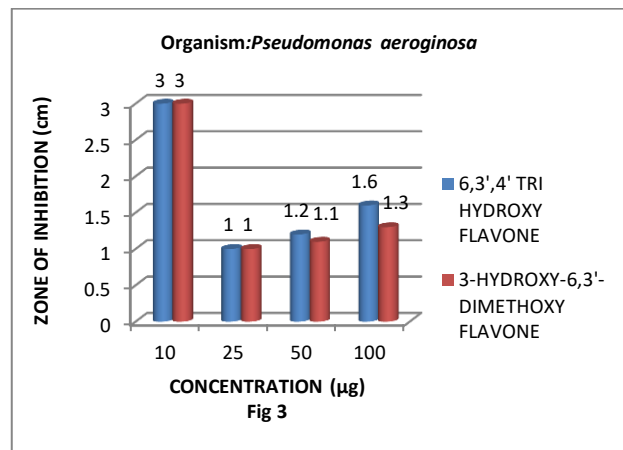
Antifungal activity

The antifungal activity was determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown species of fungus *Aspergillus niger* and *Candida albicans* was swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentration was added; the zone of inhibition was measured after overnight incubation and compared with that of standard antimycotic (Clotrimazole).

RESULTS AND DISCUSSION

Gram Negative organisms

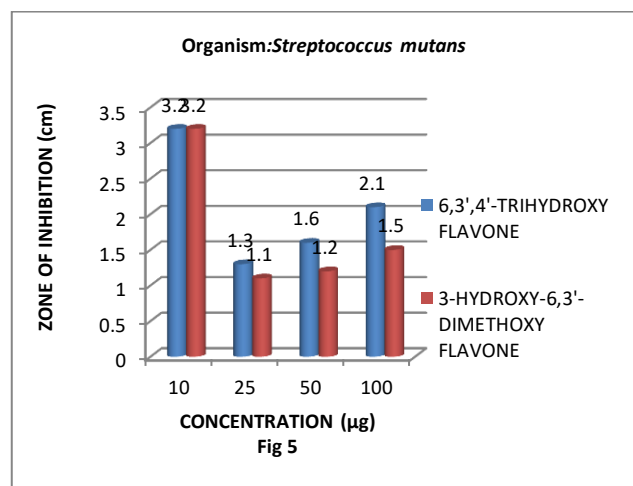
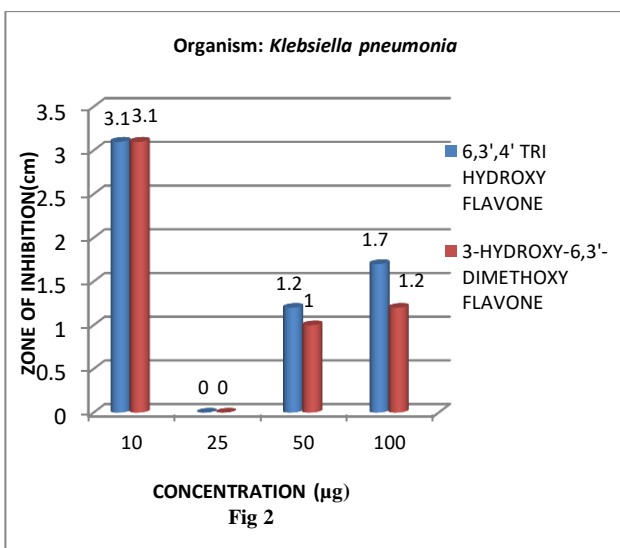
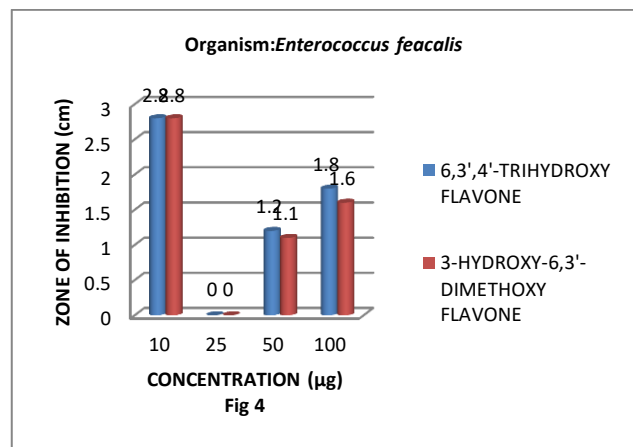
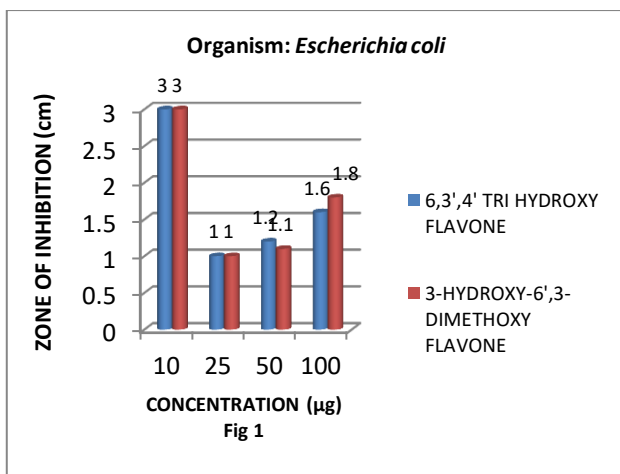
The two flavones showed inhibitory effect on the three gram negative organisms *E.coli*, *K.pneumoniae* and *P.aeruginosa*. The maximum zone of inhibition was seen with the standard drug Streptomycin at a concentration of 10µg. The two substituted flavones tested showed activities less than streptomycin as seen with decreased zone of inhibition values.

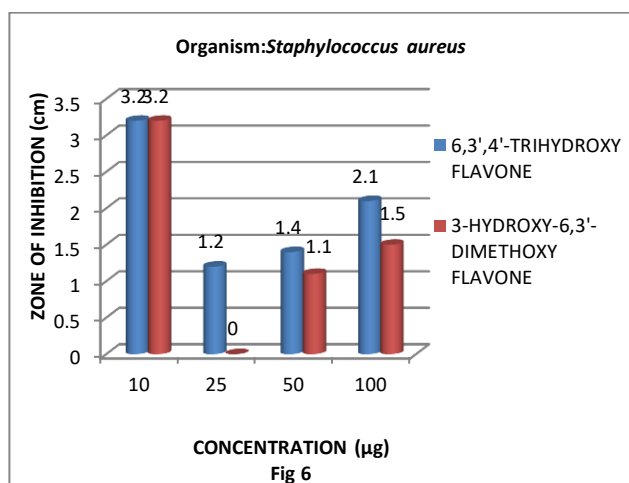


The standard drug Streptomycin gave zones of inhibition as 3 cm, 3.1cm, 3 cm at 10µg concentration against *E.coli*, *K. pneumoniae*, *P.aeruginosa* respectively. Maximum zone of inhibition values was shown by 6,3',4'-THF against the three gram negative organisms when compared to 3-hydroxy-6,3'-DMF.

Gram positive organisms

Both substituted flavones showed comparatively better inhibitory effect against the gram positive organisms *Enterococcus faecalis*, *Streptococcus mutans* and *Staphylococcus aureus*.

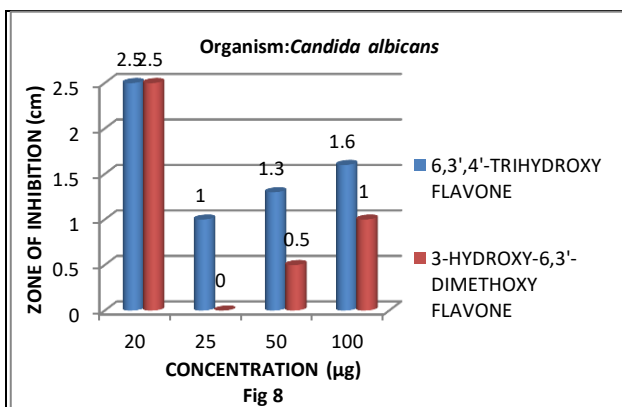
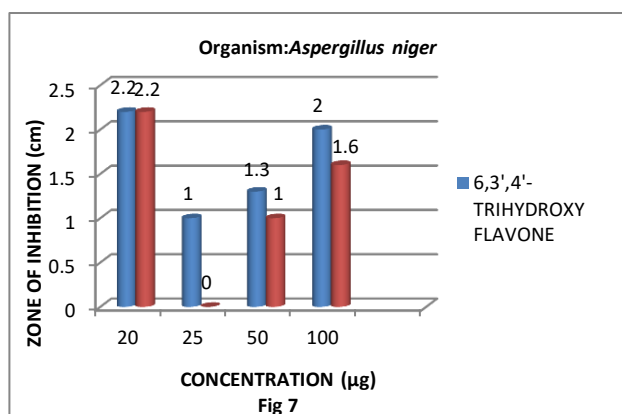




The standard drug Streptomycin gave zones of inhibition as 2.8 cm, 3.2 cm, 3.2 cm at 10 µg concentration against *Enterococcus faecalis*, *Streptococcus mutans* and *Staphylococcus aureus*. The zone of inhibition was comparatively better with 6,3',4'-THF against the three gram positive organisms, than 3-hydroxy-6,3'-DMF.

Antifungal activity

The substituted flavones exhibited varying degrees of anti-fungal activity. At 25 µg/ml, 3-hydroxy-6,3'-DMF did not exhibit any activity against both *Aspergillus niger* and *Candida albicans*. The standard drug clotrimazole gave zones of inhibition as 2.2 cm and 2.5 cm against *Aspergillus niger* and *Candida albicans* respectively.



6, 3', 4'-THF showed better inhibitory effect against *Aspergillus niger* while 3-hydroxy-6,3'-DMF gave least activity against *Candida albicans*.

CONCLUSION

This study has shown that both 6, 3', 4'-trihydroxy flavone and 3-hydroxy-6, 3'-dimethoxy flavone possess significant antibacterial and antifungal effect. Of the two compounds, 6, 3', 4'-trihydroxy flavone showed comparatively better antibacterial and antifungal effect. It showed better activity against gram positive organisms, probably due to the presence of hydroxy group in ring B, and since it is active against *Aspergillus niger*, it could be used in immune compromised patients as this fungus is shown to cause invasive disease in these patients. Thus this compound has potential to be used as effective antibacterial and antifungal agent.

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Source of Support: Nil, Conflict of Interest: None.

