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A COMPARATIVE STUDY OF BACTERIOSTATIC ACTIVITY OF SYNTHETIC HYDROXYLATED FLAVONOIDS

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ABSTRACT

Among other properties, flavonoids present a notable bacteriostatic activity. In this paper, minimal inhibitory concentrations (MICs) of 5,7,4'-trihydroxyflavanone (naringenin), 5,7-dihydroxyflavone and 2',4',4-trihydroxychalcone (isoliquitirigenin) against *Staphylococcus aureus* ATCC 25 923 were determined and compared to values obtained for other chalcones and flavanones previously investigated. Specific growth rates and MICs were determined by a turbidimetric kinetic method. The observed sequence $MIC_{flavanone}$ (inactive) >$MIC_{7\text{-hidroxyflavanone}}$ (197.6 μgml^{-1})>$MIC_{5,7,4'\text{-trihydroxyflavanone}}$ (120 μgml^{-1}) showed that the introduction of an electron donating group (-OH) causes an increase in bioactivity. On the other hand, the comparisons $MIC_{5,7,4'\text{-trihydroxyflavanone}}$ (120 μgml^{-1}) >>> $MIC_{2',4',4\text{-trihydroxychalcone}}$ (29 μgml^{-1}) and $MIC_{5,7\text{-dihydroxyflavone}}$ (105 μgml^{-1}) >>> $MIC_{2',4'\text{-dihydroxychalcone}}$ (28.8 μgml^{-1}) indicated that the chalcone structure is the most favourable for bacteriostatic activity within the flavonoid family.

Key words: flavanone, flavone, chalcone, bacteriostatic activity

INTRODUCTION

In the last years, flavonoid family members, like chalcones, flavanones and flavones, have gained increasing interest due to their numerous applications and properties. Thus, research and use of these compounds as antioxidants (2,10,16), antimutagenic (6,7) and antibacterial (1,17), as well as their vasodilator effects (9), antiallergic activity (3), antidiabetic effect (15) and antiviral action (8) are very important.

An increase in bacteriostatic action by the introduction of an electron donating group (-OH) in the aromatic A and B-rings of chalcone has been demonstrated in previous works (12,14). On the other hand, a study on trihydroxylated chalcones suggests that bacteriostatic activity is related to the number and position of hydroxyl groups (4).

In order to elucidate which structure is the most convenient for bacteriostatic action within the flavonoid family, minimal inhibitory concentrations (MICs) of 5,7,4'-trihydroxyflavanone (I), 5,7-dihydroxyflavone (II) and 2',4',4-trihydroxychalcone (III) against *Staphylococcus aureus* ATCC 25 923 were evaluated using a turbidimetric kinetic method (13).

MATERIALS AND METHODS

Microbial strain

S. aureus ATCC 25 923, mantained by sucessive subcultures in tripticase soy agar (BBL) at 4ºC and by liofilization.

Chemicals

High purity compounds were employed (Sigma 99%, purity degree): 5,7,4'-trihydroxyflavanone (I) and 5,7-dihydroxyflavone (II). 2',4',4-trihydroxychalcone (III) was prepared by Claisen-Schmidt condensation (5). KOH solution to an equimolar 4-hydroxybenzaldehyde and 2,4-dihydroxyacetophenone solution in ethyl acetate:water (1:1) was added. The reaction mixture was kept at 25ºC for five days. It was diluted with water and acidified with concentrated HCl. The total solution was treated with ethyl acetate to obtain the desired product and dried with Na_2SO_4. This

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extract was concentrated under reduced pressure at 50ºC and purified on silica gel and Sephadex LH 20 columns chromatography using benzene and methanol as eluents, respectively. After removal of methanol, the yellow-orange crystalline solid obtained yielded 15% of the pure product. The compound purity was checked by thin layer chromatography (TLC) (polyamide 11 F_{254}, methanol-acetic acid-water, 90:5:5) and spots on the plate were visualized under UV light. The structure was determined based on the chromatographic and spectroscopic data: Rf 0.174; UVλmax (MeOH) nm: 368; 242; 205; ^{1}H NMR and ^{13}C NMR (4).

Culture media

Nutritive agar (Oxoid); Müller-Hinton broth (Oxoid).

Turbidimetric kinetic method

A 24 h culture of *S. aureus* ATCC 25 923 in slant agar was transfered to 30 ml of Müller-Hinton broth and incubated 18 h at 35ºC with permanent stirring in order to be used as inoculum. Erlenmeyers containing 100 ml of culture medium with progressive concentrations of the drug to be tested were inoculated with 2 ml of inoculum and stirred in a Rosi 1000 culture chamber at 35ºC and 200 rpm, leaving one without drug as control. Aliquots were extracted at 20 min intervals during 5 h and transmittance (T) was registered in a UV-Visible recording spectrophotometer Shimadzu 160 A. The values of T were related with the number cfu.ml^{-1} (N), through the following expression (14):

$$\ln N_t = 27.4 - 10.3 \, . \, T \qquad (1)$$

RESULTS AND DISCUSSION

The compounds assayed were efficient against *S. aureus* ATCC 25 923. The number of cfu.ml^{-1} at different times was calculated by the expression of the turbidimetric kinetic method.

Considering the microbial growth law:

$$\ln N_t = \ln N_o + \mu \, . \, t \qquad (2)$$

where t: time in min; N_o: cfu.ml^{-1} for t = 0; N_t : cfu.ml^{-1} for a time t; μ: specific growth rate in min^{-1}, values for *S. aureus* specific growth rates in media with progressive drug concentrations were obtained from the ln N_t vs t plot during exponential growth phase. Results of growth of *S. aureus* in presence of 2',4',4 - trihydroxychalcone are shown in Fig. 2.

Table 1 exhibits values for the microbial specific growth rates and the drug concentrations added to the culture media.

The obtained results were interpreted satisfactorily by means of the bacteriostatic inhibition mechanism previously proposed (11). Thus, the variation of the specific growth rate (μ) with the drug concentration follows the relation

$$\mu = \mu_T - k \, . \, C \qquad (3)$$

where, μ: specific growth rate (min^{-1}); μ_T: specific growth rate in medium without drug (min^{-1}) (control); k: specific inhibition rate (ml. μg^{-1},min^{-1}); C: drug concentration (μg.ml^{-1}).

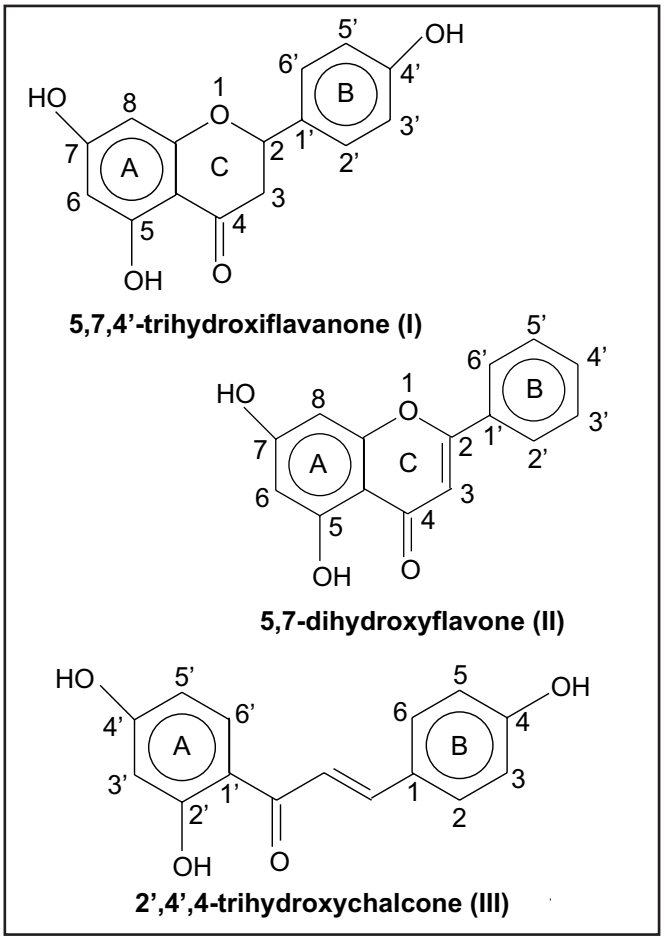

Figure 1. Structure of the flavonoids used in this study.

Table 1. Specific growth rate of *S. aureus* ATCC 25 923 as a function of the concentration of flavonoids I, II and III.

I	C	0	25.35	32.96	40.56	45.63	50.70
	μ x 10^3	50.0	39.83	37.86	32.73	30.51	29.07
II	C	0	12.30	21.54	24.61	27.70	30.77
	μ x 10^3	50.0	45.61	40.60	41.31	36.78	35.57
III	C	0	10.53	14.74	21.00	27.38	33.00
	μ x 10^3	50.0	33.02	27.73	14.86	4.60	0

C: drug concentration (μgml^{-1}); μ: specific growth rate (min^{-1}); I: 5,7,4'-trihydroxyflavanone; II: 5,7-dihydroxyflavone and III: 2',4',4- trihydroxychalcone.

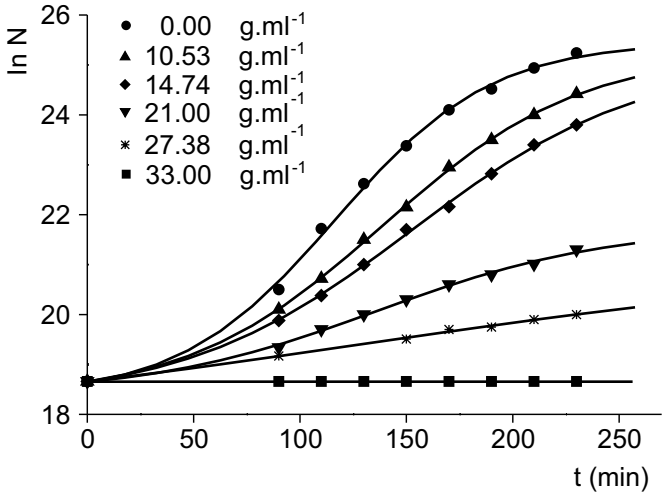

Figure 2. Growth of *Staphylococcus aureus* ATCC 25 923 in media with 2',4',4-trihydroxychalcone.

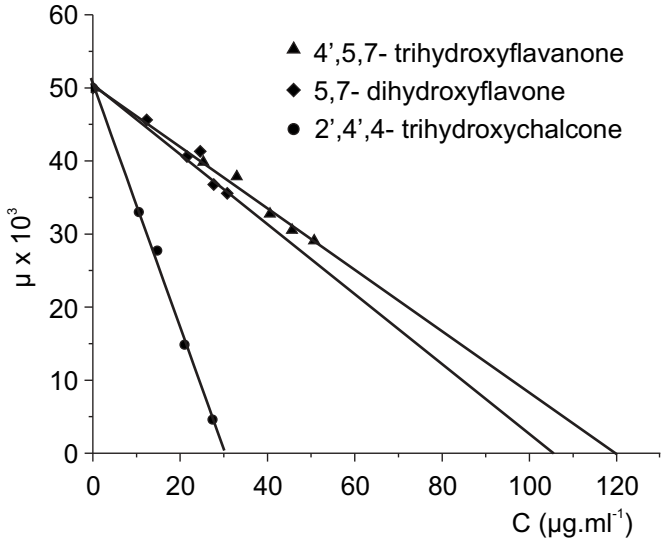

Figure 3. Graphical determination of MICs (minimal inhibitory concentrations) by extrapolation at the abcis when $\mu = 0$.

The graphical representation of expression *(3)* is shown in Fig. 3 for the assayed compound and minimal inhibitory concentrations were evaluated by extrapolation at $\mu = 0$.

Previous works (11,12,13) described the original kinetic-turbidimetric procedure and reported that MICs of 2',4'-dihydroxychalcone, flavanone and 7-hydroxyflavanone were 28.8 μgml previously 197.6 μgml^{-1}, respectively.

The minimal inhibitory concentration values obtained for the compounds assayed in our study were as follows: $MIC_{5,7,4'\text{-trihydroxyflavanone}}$ 120 μgml^{-1} >>> $MIC_{2',4',4\text{-trihydroxychalcone}}$ 29 μgml^{-1} and $MIC_{5,7\text{-dihydroxyflavone}}$ 105 μgml^{-1} >>> $MIC_{2',4'\text{-dihydroxychalcone}}$ 28.8 μgml^{-1}.

These results indicate that the chalcone structure is the most favourable for bacteriostatic activity within the flavonoid family.

Inhibition values were compared with other values previouly determined: $MIC_{flavanone}$ inactive >$MIC_{7\text{-hydroxyflavanone}}$ 197.6 μgml^{-1} >$MIC_{5,7,4'\text{-trihydroxyflavanone}}$ 120 μgml^{-1}. This sequence clearly shows that the introduction of an electron donating group (-OH) increased the bioactivity of these compounds.

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RESUMO

Um estudo comparativo da atividade bacteriostática de flavonóides hidroxilados sintéticos

Os flavonóides apresentam, entre outras, uma notável atividade bacteriostática. Neste trabalho determinaram-se as concentrações inibitórias mínimas de 5,7,4'-trihidroxiflavanona (naringenina), 5,7-dihidroxiflavona e 2',4',4-trihidroxichalcona (isoliquitirigenina) frente a *Staphylococcus aureus* ATCC 25 923 e comparadas com valores obtidos para outras chalconas e flavanonas investigadas previamente. As velocidades específicas de crescimento e as MICs foram avaliadas por um método cinético-turbidimétrico. A sequência observada $MIC_{flavanona(inactiva)}$ >$MIC_{7\text{-hidroxiflavanona}}$ (197,6 μgml^{-1}) > $MIC_{5,7,4'\text{-trihidroxiflavanona}}$ (120 μgml^{-1}) mostrou que a introdução de um grupo doador de elétrons (-OH) provoca um aumento da bioatividade. Por outro lado, $MIC_{5,7,4'\text{-trihidroxiflavanona}}$ (120 $\mu g\ ml^{-1}$) >>> $MIC_{2',4',4\text{-trihidroxichalcona}}$ (29 $\mu g\ ml^{-1}$) e $MIC_{5,7\text{-dihidroxiflavona}}$ (105 $\mu g\ ml^{-1}$) >>> $MIC_{2',4'\text{-dihidroxichalcona}}$ (28,8 $\mu g\ ml^{-1}$) permitiram concluir que a estrutura chalcona é a mais favorável para a atividade bacteriostática dentro da família dos flavonóides.

Palavras-chave: flavanona, chalcona, atividade bacteriostática

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