Antibacterial properties of quercetin

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Abstract

Quercetin is a polyphenolic flavonoid with potential chemoprotective properties. In the present work its antibacterial properties were studied against *Staphylococcus aureus, Escherichia coli, Shigella flexneri, Proteus vulgaris, Pseudomonas aeruginosa, Lactobacillus casei var shirota* by broth dilution method. Quercetin inhibited *S. aureus, P. aeruginosa* at concentration 20 mcg/mL while *P. vulgaris* and *E. coli* were inhibited at concentration 300 mcg/mL and 400 mcg/mL respectively. *Shigella flexneri* and *Lactobacillus casei var shirota* were completely indifferent even at concentration of 500 mcg/mL.

Introduction

Quercetin is a polyphenolic bioflavonoid found in fruits and vegetables. It belongs to flavonol group of flavonoids. It is known to have antioxidant, anti-artherogenic, anti-inflammatory,[1] anti-carcinogenic,[2] neuroprotection properties,[3] antibacterial,[4,5] and antiviral properties.[6] Quercetin also has GRAS (Generally Recognized As Safe) status.[7]

The spoilage and poisoning of foods by micro-organisms is a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available.[4] The consumers' acceptance for preservatives with chemical origin is decreasing; therefore the producers are looking for natural compounds which can be an alternative and supplemented to food products which will help to prolong their shelf-life and microbial safety.

In the present study effectiveness of quercetin as antibacterial agent was studied to check its potential to be used as preservative agent or its utilization in functional foods.

Materials and Methods

Quercetin Dihydrate (Sigma), Sterile Muller and Hinton Broth, Sterile MRS broth, Dimethyl sulphoxide (DMSO) (Hi Media).

Microorganisms

Staphylococcus aureus NCIM 2079, Escherichia coli NCIM 2065, Pseudomonas aeruginosa NCIM 20136, Proteus vulgaris NCIM 2027, Shigella flexneri NCIM 5265 (Procured from NCL, Pune). *Lactobacillus casei var shirota* was isolated from Yakult.

Determination of minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) of the Quercetin was determined using the broth dilution method.[8] Bacterial strains were first grown on Muller Hinton Medium for 18 to 24h at 37°C. The inoculums of the indicated bacterial strains were transferred into physiological suspension medium and adjusted to 0.5 Mac Farland turbidity standard. Quercetin stock of 10 mg/mL was prepared in 10% DMSO. St. Muller Hinton Broth was used as diluent. Dilutions were prepared in the range of 10 mcg/mL to 500 mcg/mL. 0.1 mL of bacterial inocula was added in each dilution. Growth control and sterility control were maintained. The tubes were incubated at 37°C for 24h. Bacterial growth was indicated by the presence of turbidity. All tests were carried out in triplicates.

The least concentration of Quercetin that did not permit any visible growth of the inoculated test organisms in broth culture was regarded as the minimum inhibitory concentration in each case.[9]

Determination of minimum bactericidal concentration

After culturing the test organisms separately in Mueller and Hinton broth containing various concentration of the Quercetin, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. 0.1 mL from each tube demonstrating no visible growth was removed to spread on sterile nutrient agar plate. The culture was incubated at 37°C for 24h. The lowest concentration of extract which showed no bacterial growth on solid medium after the incubation period was regarded as minimum bactericidal concentration. All samples were assayed in triplicates.

Effect of quercetin on *Lactobacillus casei* var shirota

The strain of *Lactobacillus casei var shirota* was isolated on Sterile MRS agar from commercially available probiotic drink Yakult. Incubated at 30°C under anaerobic condition.

The purified culture was preserved on sterile MRS agar slants.

The inocula of *L.casei var shirota* was prepared as described above. Effect of Quercetin on *L. casei var shirota* was performed in the similar fashion as described for determination of MIC for other bacterial cultures. For confirmation of effect of Quercetin on growth of *L. casei var shirota* agar well diffusion method was carried out. Culture suspension was prepared in physiological saline. 0.6ml of culture (OD_{550}-0.2) was added to Sterile molten MRS agar and was allowed to set. Wells were punched and each dilution of Quercetin from MIC were put into the wells. The plates were incubated at 30°C under anaerobic condition.

Results

In the present study, a good antibacterial activity of Quercetin was observed. Minimum inhibition concentration of Quercetin against the six studied bacteria ranged between 20-400 mcg/mL. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were inhibited at 20 mcg/mL while moderate activity was seen against *Escherichia coli* and *Proteus vulgaris* and no activity against *Shigella flexneri* and *Lactobacillus casei var shirota* which were confirmed by Minimum Bactericidal con-

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Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of quercetin.

Bacteria	MIC (mcg/mL)	MBC (mcg/mL)
Staphylococcus aureus NCIM2079	20	50
Escherichia coli NCIM2065	400	>500
Proteus vulgaris NCIM2027	300	>500
Shigella flexneri NCIM5265	NA	-
Pseudomonas aeruginosa NCIM2036	20	50
Lactobacillus casei var shirota	no activity	-

centration and agar well diffusion method respectively (Table 1). This results are in agreement with earlier investigations reported by other authors to inhibit different microorganisms such as *E. coli, Klebsiella pneumoniae, Bacillus cereus, A. parasiticus, A. flavus, S. aureus, S. epidermidis, B. subtilis, M. luteus and E. coli* in concentration ranging from 100-500 mcg/mL.[4] Also, in another study with Coumarin and Quercetin, antimicrobial activity of these agents against gastroenteritis bacterial strain is documented.[10]

Discussion

The antibacterial properties of Quercetin are clear from work of(Rauha JP2000; Nitiema LW2012). Also the present studies shows effectiveness of Quercetin as antibacterial agent on selected organisms. *Shigella flexeneri NCIM5265*, *Lactobacillus casei var shirota* is unaffected by Quercetin.

This interaction between Quercetin and *Lactobacillus casei var shirota* can be utilized for development of functional foods; the same is evaluated further. Environmental factors and the genetic make-up of the host can modulate the distribution of microbial strains, diet appears to be a major factor in regulating the concentration of individual species of microorganisms that colonize the gut.[11] Recent research has unveiled a potential therapeutic role of indigenous non-pathogenic microorganisms (probiotics) in maintenance of human health and treatment of various gastrointestinal diseases.[12] In addition, the use of plants for medicinal purposes has been, and remains, common practice throughout much of the world. Plants contain several bioactive compounds, including phytosterols, phytoestrogens, polyphenols and polyunsaturated fatty acids.[13] Many of these compounds have been investigated for their anti-inflammatory, antioxidant and/or anticarcinogenic properties, and have been shown to modulate numerous immunological and cellular functions.[14,15] It has been well documented that intake of flavonol rich foods can modify the composition of gut microbiota exerting pre-biotic like effect.[16,19] Unabsorbed dietary phenolics and their metabolites have been shown to exert antimicrobial or bacteriostatic effect.[17,18] These metabolites selectively inhibit pathogen growth while favouring growth of commensals bacteria and in few cases its rather found to favour growth of Lactobacillus and Bifidobacterium.[16,17] It is clear that flavanol and probiotics have beneficial effects.

Conclusions

In conclusion, the antibacterial properties of Quercetin can be used in preservation of food. Also a carefully designed, mechanistic-based laboratory and clinical studies will help in developing functional foods containing flavonols and pro-biotics.

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