Synergistic effect of ceftazidime and flavonoids against Streptococcus pyogenes

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The first person is the presenting author.

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Keywords: Synergistic effect, Ceftazidime, Flavonoids, Streptococcus pyogenes, FT-IR microspectroscopy

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| Introduction Streptococcus pyogenes is one of the most frequent pathogens of humans. S. pyogenes can infect when defenses are compromised or when the organisms are able to penetrate the constitutive defenses. It is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening invasive illness. One of the most severe invasive manifestations is streptococcal toxic shock syndrome (STSS).¹ Beta-lactam antibiotics such as penicillin and amoxicillin are uniformly effective against most strains of S. pyogenes. However, increasing antimicrobial resistance of S. pyogenes has been observed during the last decade in Europe and worldwide.² Flavonoids constitute the largest group of plant phenolics, |
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| Strain and growth conditions: S. pyogenes ATCC 19615 was used in this study and cultivated with aeration at 37 °C in Brain Heart Infusion (BHI) broth. The cell culture was centrifuged at 4,000 rpm for 10 min. The cell pellets were washed with saline (0.85% NaCl), recentrifuged and resuspended in saline. The cell was adjusted to appropriate concentration for antibiotic susceptibility testing. Drug and chemical preparations: Stock solution of ceftazidime was dissolved in distilled water and isolated flavonoids contain luteolin, baicalein and quercetin were prepared in 5% DMSO. Minimum Inhibitory Concentrations (MICs) determination: Overnight culture (18 hr.) was adjusted to give 5 x 10 ⁵ CFU/ml in BHI broth, which plus 10% serial dilutions of the ceftazidime or isolated flavonoid, Tubes of broth without antibacterial agent was used as the control. The lowest concentration of each antibacterial which inhibited growth was taken to be the MIC. ⁵⁻⁶ |
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Results

The minimum inhibitory concentrations (MICs) for the ceftazidime, luteolin, baicalein and quercetin against *S. pyogenes* ATCC 19615 are shown in Table 1. This strain was susceptible to ceftazidime, luteolin, baicalein and quercetin at MIC of 0.25 μ g/ml, 128 μ g/ml, >256 μ g/ml and 128 μ g/ml respectively. Other results include:

Checkerboard assay: The fraction inhibitory concentration of ceftazidime plus isolated luteolin, baicalein and quercetin were 0.625, <0.625 and 0.531 respectively (Table 1). The results showed that ceftazidime at concentration of 0.25 μ g/ml was significantly reduced when combined with isolated flavonoids against this strain.

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Table 1. Minimum Inhibitory Concentrations (MICs), fractional inhibitory concentrations (FICs) and FIC indexes

| S. pyogenes ATCC 19615 | MIC (µg/ml) | FIC (CF + FV) (µg/ml) | FIC index |
|------------------------|-------------|-----------------------|-----------|
| Ceftazidime | 0.25 | - | - |
| Luteiolin | 128 | 0.125 + 16 | 0.625 |
| Baicalein | >256 | 0.125 + <32 | < 0.625 |
| Quercetin | 128 | 0.125 + 4 | 0.531 |

CF = Ceftazidime; FV = Flavonoids

| querceti | n were co | onfirmed i | n viability | curve | | th luteolin, | |
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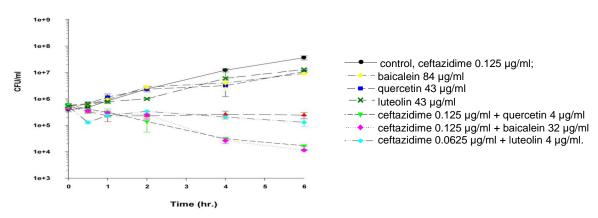


Figure 1. Viability of *S. pyogenes* ATCC 19615 after treatment with ceftazidime alone and in combined with flavonoids (the bars represent the standard deviations of three replicates)

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Discussion The results from MICs determination indicated that the isolated flavonoids inhibited S. pyogenes ATCC 19615. These results are in substantial agreement those of Banso and Mann, they found that the antibacterial test of the flavonoid fraction from *Antiaris africana* showed activity against *B. subtilis*, *S. pyogenes* and *E. coli* ¹⁰⁾. The checkerboard assay revealed that FIC indices of ceftazidime plus flavonoids were lower than 1.0 against this strain. It has been proposed that synergy can be announced when the FIC index < 1.0 11). (Arial 10, regular typeface)

| Conclusion |
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| Our findings provide evidence that all selected flavonoids have synergistic effect with ceftazidime against <i>S. pyogenes</i> ATCC 19615. The primary mechanism of action of ceftazidime plus these lavonoids is 1. Disruption of the cytoplasmic membrane function of <i>S. pyogenes</i> ATCC 19615 |
| which resulted in a loss of cytoplasmic constituents and ions. 2. Alter protein synthesis and disrupt |
| nucleic acid synthesis whereas do not effect fat synthesis. |
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