The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. Emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substance from other sources including plants\(^1\).

Natural products are a source of traditional herbal medicine and are still use in the primary health care system\(^2\). Antimicrobials of plant origin have enormous therapeutic potential\(^3\). Extracts from medicinal plants have showed the effectiveness of traditional herbs against microorganisms; as a result, plants are one of the bedrocks for modern medicine to attain new principles\(^4\). The synergistic effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment\(^5\). Therefore, the present study was undertaken to investigate synergistic activity of ethanol extract of Cassia auriculata with Tetracycline.

The flowers of Cassia auriculata were collected, thoroughly dried under shade, powdered mechanically and sieved through No.20 mesh sieve. The finely powdered flowers were kept in an airtight container until the time of use. The extraction of flower was carried out by continuous hot percolation method using 95% ethanol. The extract was concentrated under controlled temperature between 40-50°C. The percentage yield of the C. auriculata flower extract was 18.2%.

The bacterial strains used were Escherichia coli NCIM 2118, Bacillus subtilis NCIM 2010, and Staphylococcus aureus NCIM 2127. All the organisms were grown over night (24 hr) at 37°C on Nutrient Agar (NA) and transferred a loop full to 10 ml of Mueller-Hinton (MH) broth and incubated without agitation for 24 hrs at 37°C. Bacterial inoculum was standardized by matching the turbidity of the culture to 0.5 McFarland standards by diluting with fresh MH broth\(^6\).

The MICs of various drugs were determined in Muller-Hinton broth (Difco, USA) containing the same volume of medium having different concentration of plant extract. Cells in the test medium were incubated at 37°C for 24h. The MICs were determined as the lowest concentration of antimicrobial agents or the flower extract at which bacterial cells unable to grow.

Synergy testing was performed for 3 strains (S. aureus, B. subtilis, E. coli) by the checkerboard synergy method in 5 ml test tubes with MH broth. The concentrations tested for each antimicrobial typically ranged from four concentrations below the MIC to three concentrations above MIC, using 2-fold dilutions of each antimicrobial. The flower extract of C. auriculata was tested at concentrations of 128-8192 µg/ml (Drug A). Ofloxacin (Drug B) was tested at concentrations of 0.25-16 mg/ml. The procedure followed by the method described by Ghaly et al. (2009)\(^7\).
The results of checkerboard testing were interpreted by the pattern they form on the isobologram. Synergy was determined by calculating the fractional inhibitory concentration (FIC) index as follows: \( FIC = \frac{\text{MIC}_{A} + \text{MIC}_{B}}{\text{MIC}_{A} + \text{MIC}_{B}}, \) where \( \text{MIC}_{A} \) is the MIC of the organism to Drug A alone. FIC is the FIC of Drug A and FIC is the FIC of Drug B. Results of synergy testing were defined as follows: 0.5, synergy; >0.5 but <1, partial synergy; 1, additive effect; >1 but <4, indifference; >4, antagonism.

Results of minimum inhibitory concentration are shown in Table 1. The MIC of \( C. auriculata \) flower extract ranged between 1024 to 2048 mg/ml, with respect to all the test bacteria. The checkerboard technique was performed to evaluate the interaction of the floral extract in combination with ofloxacin. The FIC indices of the combination for the three bacterial strains are presented in Table 2.

The MIC of ofloxacin alone to \( S. aureus \) NCIM 2127 was 2mg/ml, while in combination with the flower extract, the MIC of ofloxacin to \( S. aureus \) NCIM 2127 became 1mg/ml, indicating a twofold decrease in MIC. With flower extract, a six fold decrease in MIC observed from 2048 mg/ml to 256 mg/ml. the FIC index of the combination was 0.625. Thus the FIC index of floral extract in combination with ofloxacin showed a partial synergistic effect against \( S. aureus \). The MIC of ofloxacin alone to \( B. subtilis \) NCIM 2010 was 4mg/ml, while in combination with floral extract, the MIC of ofloxacin to \( B. subtilis \) NCIM 2010 became 1mg/ml, indicating a fourfold decrease in MIC. With floral extract, a fourfold decrease in MIC observed from 1024 mg/ml to 256 mg/ml. the FIC index of the combination was 0.5. Therefore, the FIC index of the flower extract in combination with ofloxacin showed a synergistic effect against \( B. subtilis \).

For \( E. coli \), similar result was found where a fourfold decrease in MIC was observed when combined with the flower extract. With floral extract, a six fold decrease in MIC observed from 1024 mg/ml to 128 mg/ml. The FIC index of the combination was 0.375. The FIC index of floral extract in combination with ofloxacin showed a remarkable synergistic effect against \( E. coli \).

With an observation of a FIC index of flower extract-ofloxacin combination against \( E. coli \) NCIM 2118 exhibited better synergy when compared to other two strains. The calculated FIC indices for \( C. auriculata \) flower extract- ofloxacin combination against \( S. aureus \) NCIM 2127, \( B. subtilis \) NCIM 2010 and \( E. coli \) NCIM 2118, were depicted on isobologram, where the synergistic antibacterial effect of the flower extract was shown by a concave curve. The FIC index therefore proved significant synergism of \( C. auriculata \) flower extract-ofloxacin.

### Table 1. Minimum inhibitory concentration (MIC) values of \( C. auriculata \) flower extract

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>MIC(s) (µg/ml)</th>
<th>( C. auriculata ) flower extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S. aureus ) NCIM 2127</td>
<td>2048</td>
<td></td>
</tr>
<tr>
<td>( B. subtilis ) NCIM 2010</td>
<td>1024</td>
<td></td>
</tr>
<tr>
<td>( E. coli ) NCIM 2118</td>
<td>1024</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. FIC index of CAFE with ofloxacin against bacterial strains

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>FIC index of flower extract - ofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S. aureus ) NCIM 2127</td>
<td>0.625</td>
</tr>
<tr>
<td>( B. subtilis ) NCIM 2010</td>
<td>0.5</td>
</tr>
<tr>
<td>( E. coli ) NCIM 2118</td>
<td>0.375</td>
</tr>
</tbody>
</table>

In an attempt to formulate a new synergistic antimicrobial for bacterial strains we combined \( C. auriculata \) flower extract with ofloxacin. By checkerboard synergy technique, \( C. auriculata \) flower extract and ofloxacin exhibited synergy at sub inhibitory concentration. The exact mechanism of synergy is currently unknown but several hypotheses can be put forward to explain its mechanism of action. Firstly, if the floral extract disrupts lipopolysaccharide of bacterial strain, it may help restoring a porins channel thus facilitating the flow of ofloxacin to target sites. Secondly, it may cause negative effects on the efflux mechanism and let the sufficient concentration of ofloxacin to remain in bacterium thus helping in its increased antimicrobial activity.

Our results were consistent with previous in vitro studies which reported synergistic effects with significant reduction in the MICs of the antibiotics due to combination of antibiotics with crude plant extracts against bacterial strains. In these experiments, the change in MIC was noticed in plant extracts against ofloxacin including these plant extracts showed weak antibacterial activity. \( C. auriculata \) floral extract showed a decrease in MIC to ofloxacin and this could be referred to that these crude extracts have many different phytochemical constituents, which might inhibit bacteria by different mechanisms. This double attack of both agents on different target sites of the bacteria could theoretically lead to either an additive or synergistic effect.

### References


