Study of the Antimicrobial Activity and Synergistic Inhibitory Activity of Carica papaya leaves extract with Antibiotics on Escherichia coli

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ABSTRACT

Street food is one of the major causes of diarrhoea and gastrointestinal infections. Escherichia coli are one of the major contributors for many gastrointestinal infections. Natural sources are used to derive drugs that play striking role in the prevention and treatment of human diseases. Ayurveda has described Earandakarkati (Carica papaya) in many digestive disorders. This study was carried out to show the antimicrobial activity of Earandakarkati (Carica papaya) leaves extract alone and in combination with the antibiotics including Penicillin, Erythromycin, Piperacillin and Norfloxacin using agar strip diffusion method to see whether they produce synergistic or an antagonistic effect against street food isolated Escherichia coli and diarrhoeal ETEC strain (as a control). Carica papaya leaves extract showed antibacterial effect. The only combination of papaya leaf extract with Piperacillin resulted into a synergistic effect while the other combinations exhibited an antagonistic effect.

Key words: Antimicrobial activity, Agar strip diffusion method, Synergistic, Diarrhoeal treatment, Coliforms, Carica papaya, Escherichia coli.

INTRODUCTION

Street foods are often main etiological factor of many gastrointestinal infections. Earlier Chumber et al have studied the street foods from Pune and reported the presence of coliforms.[¹] A report on FAO in 1995 confirms the same.[²] Sheth M et al have reported the presence of coliforms in different samples of the street food called Bhel Puri.[³] The main pathogen causing gastrointestinal infection is Enteropathogenic Escherichia coli (EEC).[⁴] The strains of E. coli that has the potential to cause diarrheal illness are often seen to be associated with contaminated food and water. Earlier studies by Das M have proven the presence of E. coli in the Paani puri, the most popular street food in India.[⁵] The snack comprises of three separate constituents i.e., pani (water with spices), puri (Made from all purpose flour) and filling with moong and potato (savory filling). The food being sold roadside often is accompanied with unhygienic preparation and handling, thereby making it one of the major contributors to foodborne diseases. The primary microbiological assessment testing involves detection of Coliform bacteria thereby acting as marker for prevalence of microbial contaminants. Coliforms are indicators of faecal contamination reported by Chapin et al.

Ayurveda has unique fundamentals in treating gastrointestinal disorders. Several medicinal plants have potential to treat gastrointestinal infections. Erandkarkati (Carica papaya) is the plant that belongs
to the family Caricaceae. Several plant parts of the papaya, including the leaves, fruit, seed, latex, and root possess medicinal properties.[6] The leaves of this plant are known to increase platelet count and are reported for its antiviral properties and hence are used against Dengue where there is decline in the number of platelet count.[7] The leaves of this plant have anti-inflammatory property.[8] The leaves of the papaya plants embrace phytoactive chemical compounds of Carpaine, which is capable of killing microorganisms that often interfere with the digestive functioning.[9] Papaya leaves also contain various types of vitamins and secondary metabolites in the form of phytochemicals like; alkaloids, tannins and saponins each may have a distinctive role as antimicrobial agent. Betoni et al. (2006) illustrated that plants either exhibit antimicrobial properties to operate in synergism with antibiotics or own compounds that can sensitize a pathogen to an ineffective antibiotic. Synergism is a positive interaction results when the combined activity exhibited by two antimicrobial agents is greater than the sum of their independent effects as reported by Aiyegoro and Okob, 2009). Therefore, in this study we have made an attempt to explore antibacterial property of Erandkarkati (Carica papaya) leaf extract and also synergistic activity with other antibiotics if any.

**Materials and Methods**

The present study was carried out in Department of Microbiology, Guru Nanak Khalsa College of Arts, Science and Commerce, Mumbai, India. All the media and reagents used for microbial culture and antibiotics for Antimicrobial Susceptibility Testing were from Hi-Media Pvt. Limited, Bombay, India. Antibiotics solutions were purchased from pharmacy, Mumbai as follows:

**Pharmacy:** Noble Plus, SVS Road, Near Hinduja Hospital, Mahim (W), Mumbai-400016

a) Benzedrine Penicillin injection (Pfizer India Ltd.)

The batch number of used injection is 2207016J and the expiry date of the same is Jan 2024

b) Erythromycin Estolate Oral Suspension (Alembic Pharma Industries)

The batch number of used suspension is 2108000936 and the expiry date of the same is Jun 2023

c) Piperacillin and Tazobactam injection (ScotMed Care Pvt. Ltd.)

The batch number of used injection is 21DL07E and the expiry date of the same is Nov 2023

d) Norfloxacin Eye drops (Cipla India Ltd.)

The batch number of used suspension is A020510 and the expiry date of the same is Aug 2025

The antibiotics were specifically selected to which E.coli is resistant (Penicillin and Erythromycin) and those antibiotics to the E.coli are susceptible Piperacillin and Norfloxacin). The selection was thereby made to analyze if the combination is working against Gram negative organisms.

**Collection of Leaves**

Disease free, fresh leaves were collected from Papaya plant from Sant Tukaram Garden, Gopi Tank Marg, Citylight, Mahim, Mumbai 400016. The plant was identified and authenticated by the Department of Botany; Khalsa college, Matunga East. The leaves were cleaned and rinsed thoroughly 5 to 7 times with sterile distilled water.

**Carica Papaya Leaves Aqueous Extract Preparation**

Aqueous extract (Solid Liquid Extraction Method) was used for aqueous extraction. Fifteen leaves of the collected and washed leaves were pestle with 50 ml of distilled water to make a fine paste. This was followed by two step filtrations. In the first step, the obtained paste was filtered by passing through a muslin cloth. The filtrate obtained was passed through Whatmann filter paper No.4 in the second step to obtain a pure extract. The extract then was stored in clean glass bottles under refrigerated conditions. The prepared extract was screened for microbial growth and further the microbial free extract was used for analysis within a week of preparation and for next lot fresh extract was prepared.

**Sample Collection and Processing**

The street food; Panipuri samples were collected from 5 different vendors across Mumbai. The Panipuri samples were collected from the following vendors;
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a) Kailash Bhelpuri House, near Citylight cinema, Mahim, Mumbai-400016
b) Damodar Stall, Keluskar Road, Shivaji Park, Dadar, Mumbai-400028
c) Gupta Bhelpuri House, Mahavir Nagar, Kandivali, Mumbai-400067
d) Varsha Chaat Corner, Ghatkopar Khaugalli, Ghatkopar East, Mumbai-400077
e) Chaatwich, Sector 17, Vashi, Maharashtra, 400703

The Khatta Pani (sour soup) was initially filtered using Muslin cloth to eliminate solid particulates was used for further bacteriological analysis within 1 hour of collection.

Isolation and Identification of Coliforms from Panipuri Samples

The sample was screened for the presence of E. coli by isolating the samples on Nutrient Agar and Eosin Methylene Blue Agar. The media were prepared from a ready composition of HiMedia and was stored at refrigerated temperature for 24 hours. Isolation was carried out until pure isolates were obtained following the incubation conditions of 37°C for 24 hours that were periodically monitored using standard Gram Staining protocol. Selective bacterial colonies were maintained on Nutrient Agar slants at refrigerated temperatures and propagated monthly. The bacterial identification was carried out by performing Biochemical tests for Indole, Methyl Red, Vogues Proskauer and Simmons Citrate. The identification was confirmed using MALDI-TOF MS by comparing the peptide mass fingerprints with that of the database.

Control Organisms used for Antimicrobial screening of Plants

The test pathogen, Enterotoxigenic Escherichia coli (ETEC diarrhoeal strain) was procured from Dr. Joshi’s Central Clinical Microbiology Lab Pvt Ltd, Vashi, Navi Mumbai. The organism was re-confirmed through biochemical tests: indole, Methyl Red, Vogues Proskauer and Simmons Citrate. The procured strain was stored at refrigerated temperature and was used within 5 days of purchase.

Antimicrobial Susceptibility Testing using Disc Diffusion Method

Sterile Mueller Hinton Agar plates were swabbed with isolate obtained from Panipuri samples and obtained Enterotoxigenic E.coli strain. The cultures were suspended in sterile saline and the optical density was adjusted to 0.15. Antibiotic discs of Penicillin, Erythromycin, Piperacillin and Norfloxacin were placed in four different quadrants of plates. Activity was tested against both test organisms. In order to examine the activity of papaya leaf extract, sterile filter paper discs dipped in aqueous papaya leaf extract were used and were also tested against both test organisms. Two discs denoted as PC1 and PC2 were placed on either of the plates. The plates were incubated at 37°C for 24 hours post which zones of inhibition were measured.

Antimicrobial Synergy Testing

Strip Synergy Diffusion Method was adopted to assess the synergistic activity of papaya leaf extract with selected antibiotics against the two test organisms. For the test, Mueller Hinton Agar plates were swab with respective bacterial isolate suspensions. Sterile filter paper strips were dipped in antibiotic solution and placed horizontally on plates in centre. Second strip was dipped in papaya leaf extract and placed vertically over the first strip perpendicular to the earlier strip. Each combination of antibiotic solution and papaya leaf extract was tested against both test organisms. Plates were incubated at 37°C for 24 hrs post which wider zone of inhibition was checked for combined activity exhibited by two antimicrobial solutions employed. The calculations were performed for obtained values to determine Synergistic or Antagonistic effect exhibited by each set.

The results were analyzed with the formula below:

Synergy = A + B < C

Additive = A+B=C

Antagonistic = A+B>C

Key:
A = Average zone of inhibition for papaya leaf extract
B = Average zone of inhibition for respective antibiotic solutions
A+B = Sum of both A and B
C = Combined effect of A and B measured diagonally

**Phytochemical Screening of the Extract**

The prepared extract was subjected to centrifugation at 10,000 rpm for 15 minutes. The obtained supernatant was collected and used in the further analysis. Phytochemical screening was performed to test for Alkaloids, Flavonoids, Tannins and Saponins.

**Test for alkaloids**

Hydrochloric acid (HCl) was added to the plant extracts (3 ml) and was then placed in a water bath for 5 minutes. Then few drops of Mayer regent were added to the mixtures. Turbidity indicates the presence of alkaloids.

**Test for flavonoids**

A few drops of diluted sodium hydroid solution were added to the stock solution of *Carica Papaya* leaves extracts (0.5 ml). An intense yellow colour appeared in the plant crude extract, which then becomes colourless upon addition of a few drops of diluted sulphuric acid that show the presence of a flavonoid.

**Test for tannins**

2 ml of extracts were added in 2 ml of distilled water and stirred. To this few drops of ferric chloride solution were added. The formation of green precipitate indicate the presence tannins.

**Test for saponins**

A stock solution from each crude extract C. papaya leaves (0.5 ml) was diluted with distilled water (20 ml), and the test tube was shaken by hand for 15 minutes. The formation of foam layer on the top of the test tube showed the presence of saponins.

**Thin Layer Chromatography**

Thin Layer Chromatography was carried out for the chromatographic analysis using solvent system Chloroform: Methanol: 5% HCl in the ratio of (5:5:3) as a mobile phase. TLC runs performed were in the laboratory conditions at room temperature. The commercially available (aluminium) TLC plate coated with silica gel used as stationary phase was spotted with standard and sample and allowed to run to obtain visible spots. The TLC plate was exposed to UV light of intensity of 254 nm in UV chamber for few minutes to visualize different spot positions. The *Rf* value was calculated using the standard formula as mentioned below; for the obtained spots and compared with that of standard.

**Standard Formula:**

\[
R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}
\]

**Observations**

**Isolation and Identification of Coliforms**

In the present study total five Panipuri samples were procured from different public realm. All samples were examined for presence of bacteria using Agar streak method using Nutrient Agar and Eosin Methylene Blue Agar. EMB Agar is selective for typical coliforms resulting into metallic green-coloured colonies. Colonies obtained were screened until a pure isolate was obtained that was monitored using Gram Staining. The Gram nature of tested colony was found to be Gram negative coccobacilli. The isolate was subjected to biochemical test for Indole, Methyl Red, Vogues Proskauer and Simmons Citrate Test and was further confirmed using MALDI-TOF MS analysis. The isolate was confirmed to be *Escherichia coli*. The data for the isolation and identification of bacteria from Panipuri samples is presented in Table 1 and Figure 1.

**Table 1: Biochemical Tests for bacterial identification.**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Colony 1</th>
<th>Colony 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole Test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl Red Test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Vogues Proskauer</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing was performed for both isolated *E. coli* from Panipuri samples as well as ETEC diarrhoeal isolate using Papaya leaf extract and antibiotics like Penicillin, Erythromycin, Piperacillin and Norfloxacin by disc diffusion method. The zones of inhibition observed against standard *E. coli* ETEC strain illustrate that papaya leaf extract exhibit a zone of 25 mm while the antibiotics used as a control vehicle result into a zone of inhibition of 26 mm for Norfloxacin, 24 mm for Piperacillin, 16 mm for Erythromycin and no zone for Penicillin. Whereas against the isolated *E. coli*, papaya leaf extract demonstrates a zone of clearance of 27 mm, antibiotics like Penicillin, Erythromycin, Piperacillin and Norfloxacin result into zones of 14 mm, 10 mm, 32 mm, 32 mm respectively. The results are indicated in Figure 2 and Figure 3. Against regular *E. coli* isolated, both Piperacillin and Norfloxacin were seen to be effective with larger zone of inhibition (41mm), Whereas for ETEC strain, Norfloxacin proved to be more effective. Overall, in our study, the extract of papaya leaves was seen to be efficient against both isolated and standard bacterial diarrhoeal etiological agent; *Escherichia coli*.

Figure 2: Zone of inhibition observed for antibiotics and papaya leaf extract against *E. coli* and ETEC strain. Plates (left) indicates zone of inhibition using papaya leaf extract against isolated *E. coli* (top), ETEC strain (bottom). On (right) antibiotic discs of Penicillin, Erythromycin, Piperacillin and Norfloxacin were tested against *E. coli* (top), ETEC strain (bottom).

Antimicrobial Synergy Testing

The results obtained for Susceptibility Testing indicates a positive antimicrobial activity of papaya leaf extract against *E. coli*. This thereby affirms the effectiveness and potency of extract of papaya leaves against the test organisms and its utility in combination therapy with selected antibiotics. Antimicrobial Synergy Testing was performed using Strip diffusion method employing papaya leaf extract denoted as ‘A’ and respective antibiotic solutions denoted as ‘B’. Each combination was tested against both *E. coli* and
diarrhoeal ETEC strain. The average zone obtained against isolated E.coli for papaya leaf extract (A) was seen to be 30 mm, for Piperacillin solution (B) it was also found to be 30 mm. The combined effect when measured diagonally was calculated to be 61 mm which is greater than the summation of A and B. Similarly, against the standard ETEC strain, the combined zone of inhibition was measured to be 68 mm which is greater than the summation of A and B that was calculated to be 62 mm. The combined activity of Piperacillin with papaya leaf extract resulted into a positive synergism against E.coli strains causing mild to severe diarrhoea. The data obtained is summarized in Figure 4 and Table 2.

**Figure 4:** Antimicrobial Synergy Testing using strip diffusion method employing respective antimicrobial solutions and papaya leaf extract against E.coli and ETEC strain. (Top to bottom) Set 1 involves antagonistic combination of Penicillin with papaya leaf extract, set 2 involves antagonistic combination of Erythromycin with papaya leaf extract, set 3 involves synergistic combination of Piperacillin with papaya leaf extract, set 4 involves antagonistic combination of Norfloxacin with papaya leaf extract.

**Table 2:** Results of the synergistic activity of Carica papaya leaves extract with antibiotics. The zone of inhibition were measured at the tip of the strip for individual effect and diagonally from the centre for combined effect. Table represents the interpretation of the values obtained for A+B and C. Set 3 with Piperacillin and papaya leaf extract exhibit synergistic effect against both test organisms while other sets demonstrate an antagonistic effect.

<table>
<thead>
<tr>
<th>Synergy Antimicrobial combination</th>
<th>E. coli</th>
<th>ETEC strain</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A+B</td>
<td>C</td>
<td>A+B=C</td>
</tr>
<tr>
<td>Papaya extract Penicillin antibiotic leaf +</td>
<td>60 mm</td>
<td>44 mm</td>
<td>46 mm</td>
</tr>
<tr>
<td>Papaya extract Erythromycin antibiotic leaf +</td>
<td>66 mm</td>
<td>53 mm</td>
<td>60 mm</td>
</tr>
<tr>
<td>Papaya extract Piperacillin antibiotic leaf +</td>
<td>60 mm</td>
<td>61 mm</td>
<td>62 mm</td>
</tr>
<tr>
<td>Papaya extract Norfloxacin antibiotic leaf +</td>
<td>64.5 mm</td>
<td>60 mm</td>
<td>57 mm</td>
</tr>
</tbody>
</table>

**Qualitative Detection test for Phytochemicals**

The pharmacological properties of *C papaya* lies in the various chemical constituents it contains. The correlation of the synergistic effect observed to the phytochemical constituents in the papaya leaf extract was determined by subjecting the extract to Qualitative Detection method. The tests indicate that the extract shows presence of secondary metabolites like Alkaloids, Tannins and Saponins each having an antimicrobial activity, while Flavonoids were seen to be absent. The results are summarized in Table 3.

**Table 3:** Phytochemical screening of the extract. The extract was subjected to phytochemical analysis for establishing correlation between the activity exhibited and the phytoactives present. The qualitative tests determine the presence of alkaloids, tannins and saponins while flavonoids were seen to be absent.
Key:
+ indicates presence of metabolite
- indicates absence of metabolite

<table>
<thead>
<tr>
<th>Secondary Metabolite</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

Thin Layer Chromatography

The chromatographic analysis for the papaya leaf extract was carried out using Thin Layer Chromatography. The mobile phase used was Chloroform: Methanol: 5% Hydrochloric acid in a ratio of 5:5:3. The spots of sample and Atropine as standard were applied to the stationary phase and was allowed to run which resulted into separation and band formation that was visualized directly and under UV chamber. The Retention factor values were calculated using the standard formula and it was observed that the values for standard and sample were in a close proximity that confirms the presence of alkaloids in the sample. The results are depicted in Figure 5 and Table 4.

Table 4: Retention factor (Rf) values for standard and sample. The Chromatogram developed was used to calculate Rf values for sample and standard. The obtained value for standard and sample lie close indicating presence of alkaloids in the sample.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine (as standard for alkaloids)</td>
<td>0.97</td>
</tr>
<tr>
<td>Papaya leaf extract (spot 1)</td>
<td>0.95</td>
</tr>
<tr>
<td>Papaya leaf extract (spot 2)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

RESULTS

A total of five samples of Panipuri were analyzed. All the samples were testes for the presence of Coliforms and pathogen identification and then result was interpreted. All the samples were examined for coliform presence by isolation on selective Eosin Methylene Blue Agar and Nutrient Agar. All of the Pani samples were seen to be contaminated with varied bacteria. The coliform isolated was identified using biochemical test and identification was confirmed using MALDI-TOF analysis and was identified to be Escherichia coli.

A standard ETEC diarrhoeal strain was used for a comparative analysis. Coliform and the ETEC strain were used for Antimicrobial Susceptibility and Synergy Testing. Susceptibility Testing indicates affirmative results for papaya leaf extract using disc diffusion method. The Synergy Testing using strip diffusion method against both the test organisms employed combinations of selected antibiotic solutions individually with papaya leaf extract. The results demonstrate the synergistic activity of Piperacillin with papaya leaf extract. The results emphasize the potency of papaya leaf extract in enhancing the activity of Piperacillin when used in combination.

The activity of Carica papaya leaf extract and the phytochemical constituents for the positive synergism was demonstrated using Qualitative detections of phytochemicals that exhibited presence of alkaloids, tannins and saponins. The alkaloid confirmation was
demonstrated using Thin Layer Chromatography that resulted into Rf values for standard and sample in a close proximity indicating alkaloid presence in the sample. The results illustrate the potential for the combination therapy of Piperacillin with papaya leaf extract against mild to severe diarrhoeal etiological agents.

**DISCUSSION**

Escherichia coli is a common tenant of the normal bacterial foliage of the gut.[11] As long as these bacteria don't acquire inheritable rudiments garbling for acidity factors, they remain benign commensals. Strains that acquire bacteriophage or plasmid DNA that is specifically involved in synthesizing enterotoxins or invasion factors become virulent and can beget either a plain, watery diarrhoea or an inflammatory dysentery. These conditions are most familiar to Westerners as Traveller’s diarrhoea. Such scenarios are also reported in endemic countries, especially common in infants. Diarrhoeal conditions are often associated with three major groups of Escherichia coli strains, those producing enterotoxins are called *Enterotoxigenic E. coli (ETEC).*[12] Street food preparations in India do not follow any FSSAI standard norms. Hence, there are more gastrointestinal infections found in street food consumers. Paani Puri is the most favourite street food in India. It comprises of Paani (water) with dates, jaggery and spices and the puri is filled with mashed potato and the spiced water. The water when tested for presence of contaminants often is tested positive for coliform bacteria. Yadav et al in their study also have confirmed the same.[13]

Ayurveda emphasizes the role of digestive fire and metabolism in the origin of all diseases.[14] *Erandkarkati (C. papaya)* is mentioned in ancient Vedic texts as *Agnideepak* meaning which enhances digestive enzymes.[15] Any infection is associated with inflammation.[16] *Carica papaya* is also known to possess anti-inflammatory properties.[17] The anti-inflammatory property has been proven experimentally proven by where extract of leaves of papaya showed significant reduction in the paw edema in the carrageenan test.[18] Earlier Atta F have proven antibacterial property of *Carica papaya* leaves.[19] Also Ajiboye et al in their study have found potential antimicrobial property of *Carica papaya*.[20] Earlier Unazae et al. have found antibacterial activity of *Carica papaya* leaf extract in *E. coli.*[21] We found phytoactives as Alkaloids, Tannins and Saponnins, (Table 3). Alkaloids are known to have antibacterial property. Thin Layer Chromatography was performed focusing on target alkaloid indicating the presence of Carpaine. (Table 4). The antimicrobial activity of *Carica papaya* leaves is probably due to the alkaloids and Carpaine.

**CONCLUSION**

Antimicrobial Strip Synergy Method resulted affirmative and a synergistic inhibition was observed for the tested coliforms using papaya leaf extract-Piperacillin combination whereas, other combinations tested although inhibited but resulted into antagonistic effect which can possibly result into resistance to organisms. The results were confirmed by synergy calculations statistically. The presence of secondary metabolites particularly that of alkaloids support the antibacterial study. Hence it can be concluded that the above stated combination of *Carica papaya* leaf extract with Piperacillin is promising to combat diarrhoea causing coliforms.

This research however, is not only restricted to the antibacterial activity but extended in exhibiting a positive synergistic effect of the papaya leaf extract with selected antibiotics against pathogenic strains of *E.coli.* The research also focuses on phytochemical analysis, the results of which are in accordance to the existing research that can thereby be correlated to the antibacterial property of papaya leaf extract.

Antimicrobial resistance is a major concern in medicine. It is a threat that is leading to deaths of millions of people across the globe.[22] There are different strategies which are planned to combat these pathogens. It has been illustrated that plants either exhibit antimicrobial properties to operate in synergism with antibiotics or own compounds that can sensitize a pathogen to an ineffective antibiotic.[23] Synergism is a positive interaction results when the combined activity exhibited by two antimicrobial
agents is greater than the sum of their independent effects. Combination therapy exhibits an expanded antimicrobial spectrum to prevent the emergence of resistant mutants, to reduce the antibiotic toxicity and to obtain a synergistic activity.

A further study can be taken up against antimicrobial resistant strains of E.coli. Though this is very limited research; it can provide a lead for further work in this area.

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