

ORIGINAL RESEARCH

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Synergistic effect of *Coriandrum sativum* L. extracts with cefoxitin against methicillin resistant *Staphylococcus aureus*, extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae***Nilay Ildiz¹, Ayse Baldemir², Ufuk Ince¹, Selen Ilgun², Yusuf Konca³**¹Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Kayseri, Turkey²Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Kayseri, Turkey³Erciyes University, Faculty of Agriculture Department of Animal Science, Kayseri, Turkey

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Abstract

Antibiotic resistance has become a general health problem that makes the treatment decisions of clinicians more difficult. Recently, plants and their compounds have been suggested as a potential alternative to antimicrobials. The present study was carried out to evaluate for the first time, possible synergistic interactions on the antibacterial efficacy of *Coriandrum sativum* L. seed extracts and cefoxitin in combination against three important nosocomial pathogens (methicillin resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae*). The antibacterial effect studied using the disc diffusion and synergism was showed by checkerboard methods. In the disc diffusion method, combinations of both methanol (ME, 1250 µg/mL) and petroleum ether extracts (PE, 1250 µg/mL) with cefoxitin (30 µg/mL) showed an increase in antibacterial activity against all tested microorganisms. It was found that, combinations of Coriander seed ME and PE extracts with cefoxitin have synergistic interactions against ESBL positive *K. pneumoniae* at 0.03516 and 0.03125 Fractional inhibitory concentration (FIC) index (FICI), respectively. The FICI of combinations against MRSA and *E. coli* were found to be indifferent by the checkerboard method. An antagonistic effect was not found in these combinations. The current study clearly suggests the potential usage of Coriander seed extracts alone and in combination with cefoxitin for combating infections by ESBL positive *K. pneumoniae* strains

Keywords: *Coriandrum sativum*, cefoxitin, *enterobacteriaceae*, MRSA, synergism, coriander**Introduction**

Antibiotic resistance has become a general health problem that makes the treatment decisions of clinicians more difficult. Recently, plants and their compounds have been suggested as a potential alternative to antimicrobials [1]. While ~80% of the drugs are derived from plants, only a few of them are antimicrobial agents [2]. Secondary metabolites in plants have a wide range of antimicrobial properties [3]. In this context, diversity of the phytochemical structures and pharmacological properties of plant / plant active components and antibacterial drug interactions may be a solution in the search for new therapeutic agents [4]. Some studies reported that the combination of plant compounds with some therapeutic agents rather than just using the compounds alone can provide synergistic effect on resistant microorganisms. Hence, this synergism may help in the provision of more effective treatment [5,6].

Coriandrum sativum L. (Coriander) is an annual herb belonging to

the Apiaceae family. In Turkey, Fructus Coriandri are traditionally used for medicinal purposes such as loss of appetite, carminative, dyspeptic and digestive complaints [7]. Previous studies have shown that Coriander has shown variety of pharmacological properties including antibacterial effects [8,9]. More recently, the essential oil (EO) components of plants showed a synergistic antibacterial effect with antibiotics [10,11]. During the last decade, several reports have confirmed the effect of plants combinations with antimicrobial agents [12,13]. Antibiotic insufficiency develops day by day in resistant strains and leads to an increase in the turnover of plant extracts that have been proven to have antibacterial effects in conventional medicine.

Extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae* (ESBL- E) infections have become a major public health problem around the world [14-16]. *Staphylococcus aureus* which is normally found in the nasal cavity, can cause minor to potentially life threatening invasive diseases and nosocomial infections. Methicillin resistant *S. aureus* (MRSA) is posing a major challenge for treatment options almost all clinically available antibiotics, such as cefoxitin which is a second-generation cephalosporin and has strong in vitro activity against ESBL [17,18]. Additionally, cefoxitin and oxacillin are employed

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for the detection of MRSA according to the Clinical & Laboratory Standards Institute: CLSI guidelines by disc diffusion methods. The mecA mediated resistance to oxacillin can be detected by using the cefoxitin or oxacillin disc diffusion method but using cefoxitin is preferred more due to it is easier to read and it also acts as an inducer of the mecA gene [19]. The antibacterial activities of individual Coriander extracts and a combination of Coriander extracts with cefoxitin were evaluated to determine synergism against MRSA, ESBL positive *Escherichia coli* and ESBL positive *Klebsiella pneumoniae* by the disc diffusion and checkerboard methods.

Material and Methods

Plant material

Coriander cultivar seed was obtained from the Agricultural Faculty of Erciyes University it was harvested in 2014 and stored in normal room conditions.

Bacterial strains

Clinical isolates (MRSA, ESBL producing *E. coli* and *K. pneumoniae*) were identified from urine specimens and collected from the microbiology department of Necip Fazıl City Hospital, Kahramanmaraş Turkey. The identification of microorganisms and sensitivity testing was performed on an automated system (VITEK® 2: Healthcare | bioMérieux). The double-disc synergy test was used in order to screen their production of ESBLs.

Preparation of the Coriander seed extracts

To prepare the ME extract of Coriander seed, a 10 g sample was weighed and ground then 100 ml methanol was added and mixed with a magnetic stirrer (Jeio Tech, MS-53M) for 8 hours. This process was repeated three times at room temperature conditions (24°C).

To prepare the PE extract of coriander seed, a 10 g sample was weighed and ground then 100 ml PE was added and treated in an ultrasonic bath filled with ice water for 15 minutes. This was repeated three times. Afterwards, the plant extracts were filtered and both of them were evaporated to dryness (Heidolph®, Heizbad-Hei-VAP). The samples were stored at -20°C.

Determination of antimicrobial activity

Susceptibility screening of microorganisms was performed according to CLSI standards by disc diffusion and the minimum inhibitory concentration method. PE and ME extracts were dissolved in 1% dimethyl sulfoxide (DMSO) and a 1250 µg/mL standard was prepared for the disc diffusion method. Sterile blank discs were used to impregnate 20 µg/mL of plant extract that was taken from the stock solution then carefully dried. Cefoxitin and cefoxitin discs (30 mcg, Bioanalyse, Turkey) are commercially available. Bacteria cultures were transferred on to Mueller-Hinton agar (Hi-Media, India) and incubated for 18 hours at 37°C. Then, the plates were examined for the presence of inhibition zones. The corresponding diameter was reported in millimeters. The MIC values of the bacteria were determined by a VITEK® 2 system (bioMérieux) for cefoxitin. The MICs were determined by the broth microdilution method according to CLSI guidelines for the extract and extract + cefoxitin combination. Final inoculums of bacteria were 2×10^6 CFU/ml and these were added to 96 well microtiter plates with 100 µl of extract/ extract+ cefoxitin

combination in serial dilution. Plates were incubated at 37 °C for 24 h. The MICs were determined visually. All assays were performed in duplicate. Bacteria in Mueller- Hinton (Hi-Media, India) broth were used as a growth control.

Synergy interaction assay

Different interactions (synergistic, additive and indifferent) can be observed by the checkerboard method. The method was used to determine synergism [20,21]. between the cefoxitin and extracts. MICs were defined by the automated VITEK® 2 system (bioMérieux) and broth microdilution method. The fractional inhibitory concentration (FIC) index was calculated according to Pei et al., 2009 [22]. The calculations used are listed below [20,23,24].

$$FIC_A = (\text{MIC specimen A in the presence of B}) / (\text{MIC specimen A individually})$$

$$FIC_B = (\text{MIC specimen B in the presence of A}) / (\text{MIC specimen B individually})$$

$$FIC \text{ Index} = FIC_A + FIC_B$$

An FIC index ≤ 0.5 means synergism determined;

An FIC index > 0.5 but ≤ 4.0 means indifference determined;

An FIC > 4.0 means antagonism shown

Results

The effects of Coriander seed extracts, cefoxitin and extract + cefoxitin on clinically resistant strains (MRSA, *E. coli*, *K. pneumoniae*) are shown in Tables 1 and Figure 1. These pathogen microorganisms were chosen due to their importance in several infections such as urinary tract infection, endocarditis, septicemia, osteomyelitis and hospital-acquired infection and have been reported to be antibiotic resistant.

Table 1. Antibacterial activity of coriander seed extract, cefoxitin and their combination on clinically resistant strains

Extract and combinations	Clinic isolates		
	MRSA	E. coli	Klebsiella pneumoniae
1250 µg/ml coriander extract, ME	8.33 ^c	8.00 ^d	8.67 ^d
1250 µg/ml coriander extract, PE	9.67 ^c	8.67 ^d	25.33 ^b
Cefoxitin, 30 µg	16.67 ^b	19.33 ^c	15.33 ^c
Cefoxitin+ 1250 µg/ml coriander extract (ME)	19.67 ^a	21.33 ^b	24.67 ^b
Cefoxitin+ 1250 µg/ml coriander extract (PE)	22.33 ^a	24.33 ^a	33.67 ^a
SEM	0.333	2.844	0.494
Probability, p	**	*	**

a,b,c,d. Values with different superscript in a column differ significantly, ***:p<0.05, **:p<0.01; SEM: pooled standard error of means

In disc diffusion assay, the ME, PE extracts and cefoxitin inhibition zones of the MRSA strain were identified as 8, 10 and 18 mm, respectively. However, the combination of extracts with cefoxitin were increased and inhibition zones were determined as 21 mm for the cefoxitin+1250 µg/ml ME extract and 22 mm for the cefoxitin+1250 µg/ml PE extract. Comparable results were also obtained for the *E. coli* isolate. Both combinations showed an increase in antibacterial activity in all tested microorganisms. However, according to the FIC results, there was no synergistic

interaction on MRSA and *E. coli* by the checkerboard method. Cefoxitin showed a small zone of inhibition, but when used with plant extracts (especially with PE extract), it exhibited a larger zone of inhibition against ESBL positive *K. pneumoniae*. The inhibition zones of *K. pneumoniae* were determined as 15 mm for cefoxitin; 9 mm for the ME extract; 25 mm for the PE extract; 25 mm for the cefoxitin + 1250 µg/ml ME extract and 34 mm for the cefoxitin + 1250 µg/ml PE extract. The susceptibility of *K. pneumoniae* increased with cefoxitin + 1250 µg/ml PE extract and inhibition zone was measured as 34 mm. According to the interaction results of the checkerboard method, *K. pneumoniae* showed synergistic interaction against combinations of cefoxitin ME and PE extracts at 0.03516 and 0.03125 FIC index, respectively.

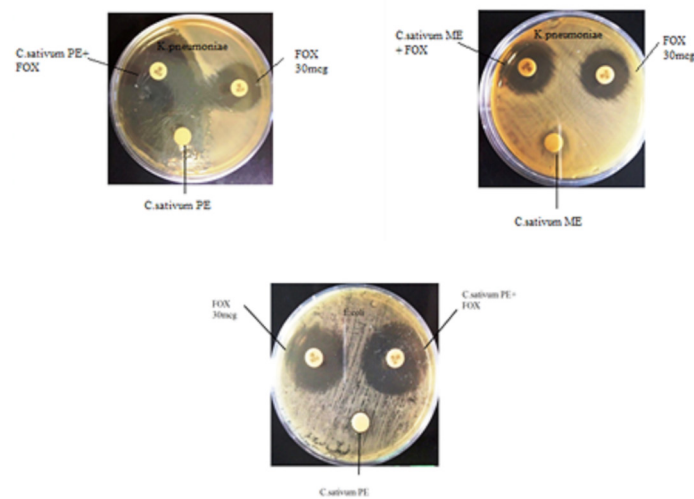


Figure 1. Inhibition zones of cefoxitin, Coriander extracts and their combinations against clinic isolates, (A) Inhibition zones of cefoxitin, Coriander PE extracts and their combinations against *K. pneumoniae* (B) Inhibition zones of cefoxitin, Coriander ME extracts and their combinations against *K. pneumoniae* (C) Inhibition zones of cefoxitin, Coriander PE extracts and their combinations against *E. coli*

Discussion

Nowadays, antibiotic resistance is increasing globally and at a frightening level [25]. The effectiveness of antimicrobial drugs for the treatment of resistant bacteria infections is limited. New antimicrobials can be developed for suppressing resistant mutants, but this is a difficult and long process. Extending the life of current antimicrobials might be possible if they were used in combination with natural products against resistant microorganisms.

Synergistic combinations could represent therapeutic alternatives for the treatment of resistant pathogenic microorganisms [26,27]. In the last decade, the frequency of antibiotic resistant strains of ESBL producing *K. pneumoniae* has increased remarkably [14-16]. Plasmid-mediated AmpC enzymes as a serious problem have been revealed in enteric bacteria. These enzymes have caused nosocomial outbreaks and treatment failure against cephalosporins and also increased mortality [28]. In the future, treatment problems due to the spread of resistant strains may arise. The current findings showed that the combination of cefoxitin with coriander extracts is effective in resistant strains (Table 2). This combination may contribute to the treatment of the ESBL producing strain.

More recently, "Plantaricin CS" namely a novel antimicrobial

peptide with wide antibacterial activity, was isolated from Coriander leaf extract. The new peptide showed effective germicidal effects on *K. pneumoniae* (MIC = 2.65 mg/mL) [11]. In the present study, the synergistic effect of Coriander seed extracts against *K. pneumoniae* may be due to the active components [29] in the ME and PE extracts.

The PE extract + cefoxitin combination was found to be twice as effective as the ME extract + cefoxitin combination against the clinically resistant *K. pneumoniae* strain (Figure 1) by the disc diffusion method. The inhibition zones of the tested extracts against MRSA were lower than those against *E. coli*. The combination of cefoxitin with coriander extracts had stronger effect than cefoxitin alone on each resistant strain. This synergistic behavior was more visible in the clinically resistant *K. pneumoniae* strain. Cefoxitin showed a synergistic effect when combined with Coriander extracts on resistant *K. pneumoniae* by the checkerboard method. The difference in the results between the two methods may be due to the deficiencies of the disc diffusion method. In studies with such plant extracts, the MIC value is essential [11]. In this study, the disc diffusion method was performed for preliminary assessment of the synergistic effect. MRSA and *E. coli* synergism values were also found as indifferent near the borderline. For this reason, we think that the checkerboard method is a more sensitive method for evaluating synergistic interaction with MIC value.

The most common cause of resistance to extended-spectrum β -lactam antibiotics in Enterobacteriaceae is the enzymatic degradation of antibiotics [30]. According to the literature, some of the mechanisms adopted in combinations are as follows: protective enzymes and common biochemical pathway inhibition, combinations of cell wall-active agents, and use of cell wall active agents to enhance the uptake of other antimicrobials [23,31]. Linalool, which is the main component of Coriander, causes permeability alteration of the outer membrane, alteration of cell membrane function and leakage of intracellular materials [29]. One of the efficient mechanisms may be that these enzymes which are responsible for the production of ESBLs, are excreted during bacterial content excretion. Similar mechanisms may also apply for plant and antibiotic combinations. The decline in antibiotic resistance with a combination of plant extracts and conventional antimicrobials has not yet been fully elucidated.

Conclusion

Antibiotic drug development is a rigorous, cost-effective and time-consuming process. New methods to reduce the development of antibiotic resistance by pathogenic organisms in the pharmaceutical industry are needed. To the best of our knowledge, this is the first report on the synergistic antibacterial activities of Coriander extracts and cefoxitin combination. This study results demonstrated that the Coriander seed extracts and cefoxitin combination has strong synergistic antibacterial activity against ESBL producing *K. pneumoniae*. These synergistic interactions may increase the antibacterial efficacy of antimicrobials at low concentrations which may reduce their side effects. Combinations of antibiotics with plant extracts can be used as potential natural antibacterial agents in the pharmaceutical industry.

Competing interests

The authors declare that they have no competing interest

Financial Disclosure

The financial support for this study was provided by the investigators themselves.

Ethical approval

Before the study, permissions were obtained from local ethical committee.

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