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Investigation of Antibacterial Activity of Curcumin and Synergistic Effect with Gentamicin Sulfate

Kurkuminin Antibakteriyel Aktivitesinin ve Gentamisin Sülfat ile Sinerjistik Etkisinin Araştırılması

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ABSTRACT

Aim: In this study; it was aimed to examine the antibacterial activities and synergistic effects of curcumin, a phytotherapeutic agent, and gentamicin sulfate on bacteria.

Materials and Methods: Antibacterial activity of different concentrations of curcumin and gentamicin sulfate on *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 3851, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 25923 minimal inhibitory concentration (MIC) and tested by disc diffusion methods. The synergistic effects of combinations of curcumin and gentamicin sulfate were examined by checkerboard test.

Results: It was found that antibacterial activity was seen in all bacteria and the lowest MIC was 7.81 µg/mL in *E. faecalis* for curcumin and 0.08 µg/mL in *K. pneumoniae* for gentamicin sulfate. As a result of the disk diffusion test, inhibition zone diameters were detected at concentrations of 32 and 16 mg/mL in all bacteria tested. As a result of the checkerboard test, an additive effect was observed in four of the bacteria (*P. vulgaris*, *B. cereus*, *L. monocytogenes*, *S. aureus*) and a indifferent effect was observed in three of them (*P. aeruginosa*, *K. pneumoniae*, *E. faecalis*). The finding of the lowest fractional inhibitor concentration index (FICI=0.75) in *B. cereus*, one of the gram-positive bacteria, was interpreted as the combination of curcumin and gentamicin sulfate having a partial synergistic effect.

Conclusion: This study is the first to evaluate the synergistic effect of curcumin and gentamicin sulfate. Antibacterial activity results suggest that curcumin can be used as an alternative agent in the treatment of bacterial infections. However, in order to clearly determine the effect of both the antibacterial activity of curcumin and its synergy with gentamicin sulfate during the treatment process, these results need to be supported by large-scale *in vitro* and *in vivo* studies that will include clinical isolates.

Keywords: Curcumin, gentamicin sulfate, antibacterial activity, synergistic effect, checkerboard test

ÖZ

Amaç: Bu çalışmada; fitoterapötik bir ajan olan kurkuminin ve gentamisin sülfatın bakteriler üzerine antibakteriyel aktiviteleri ile sinerjistik etkilerinin incelenmesi amaçlandı.

Gereç ve Yöntem: Kurkumin ve gentamisin sülfatın farklı konsantrasyonlarının *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 3851, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644 ve *Staphylococcus aureus* ATCC 25923 üzerine antibakteriyel aktivitesi minimal inhibitör konsantrasyon (MİK) ve disk difüzyon metodları ile test edildi. Kurkumin ile gentamisin sülfatın kombinasyonlarının sinerjistik etkileri dama tahtası testi ile incelendi.

Bulgular: Antibakteriyel aktivitenin tüm bakterilerde görüldüğü ve en düşük MİK'in kurkumin için *E. faecalis*'te 7,81 µg/mL, gentamisin sülfat için ise *K. pneumoniae*'da 0,08 µg/mL olduğu bulundu. Disk difüzyon testi sonucu test edilen tüm bakterilerde 32 ve 16 mg/mL konsantrasyonlarda inhibisyon zon çapı tespit edildi. Dama tahtası testi sonucu bakterilerin dördünde (*P. vulgaris*, *B. cereus*, *L. monocytogenes*, *S. aureus*) additif,

Note: This study constitutes the master's thesis of the primary author entitled 'Investigation of Antibacterial Activity of Curcumin and Synergistic Effect with Gentamicin Sulfate' within the Medical Microbiology Program at Tekirdağ Namık Kemal Institute University of Health Sciences.

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üçünde (*P. aeruginosa*, *K. pneumoniae*, *E. faecalis*) indiferans etki görüldü. Gram-pozitif bakterilerden *B. cereus*'ta en düşük fraksiyonel inhibitör konsantrasyon indeksinin (FİKİ=0,75) bulunması kurkumin ile gentamisin sülfat kombinasyonunun kısmi sinerjistik etkili olduğu şeklinde yorumlandı.

Sonuç: Bu çalışma kurkumin ile gentamisin sülfatın sinerjistik etkisinin değerlendirildiği ilk çalışmadır. Antibakteriyel aktivite sonuçları, kurkuminin bakteriyel enfeksiyonların tedavisinde alternatif bir ajan olarak kullanılabileceğini düşündürmektedir. Ancak hem kurkuminin antibakteriyel etkinliğinin hem de gentamisin sülfat ile sinerjisinin tedavi sürecindeki etkisini net olarak belirlemek adına bu sonuçların klinik izolatların dahil edileceği geniş ölçekli *in vitro* ve *in vivo* çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: Kurkumin, gentamisin sülfat, antibakteriyel aktivite, sinerjistik etki, dama tahtası testi

INTRODUCTION

Bacterial infections are one of the major causes of chronic infections and mortality. Antibiotics used in the treatment of these infections are preferred due to their potent effects. However, it is also known that the widespread use of antibiotics leads to the emergence of multidrug resistant (MDR) bacterial strains[1]. In recent years, the increase in infections caused by resistant strains has attracted attention. MDR bacteria show resistance to three or more classes of antibiotics. High morbidity and mortality rates are observed in diseases caused by these bacteria[2].

In order to prevent the increase in antibiotic resistance, there is a need for antimicrobial compounds that can be used as an alternative to conventional antibiotic therapy. This has led to the discovery of new natural or synthetic antimicrobial compounds[3]. The side effects of synthetic drugs have led to a growing interest in natural plant-derived antimicrobial agents and a growing interest in treating infections naturally[4]. Natural products are also being investigated in combination therapies to manage antibiotic resistance. Synergistic studies are expected to be important in the future to overcome antimicrobial resistance[5].

Curcumin is a food spice that is a natural component of *Curcuma longa* (turmeric, turmeric) rhizomes. It has been widely used as a medicine in the treatment of various diseases in Asian and Middle Eastern countries for years[6]. Curcumin, also known as turmeric, has been shown to have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and anticancer activities[7-9]. In the society, it is known to be used for therapeutic purposes against various malignant diseases, diabetes, arthritis, gastritis, urinary tract infections, skin diseases and other chronic diseases[10]. Studies have shown that combinations of curcumin with different agents, including various antibiotics, have synergistic effects against bacteria[11-13].

The aim of this study was to determine the antibacterial activities of curcumin and gentamicin sulfate, an antimicrobial drug, against various Gram-positive and Gram-negative bacteria. It was also planned to investigate the synergistic effects of gentamicin sulfate, which is used in clinical applications, when combined with a phytotherapeutic agent such as curcumin as well as its effects alone.

MATERIALS AND METHODS

This study was reviewed and approved by Tekirdağ Namık Kemal University Non-interventional Clinical Research Ethics Committee (approval no: 2022.68.04.18, date: 26.07.2022).

Bacteria Strains

Gram-negative bacteria of *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 3851 and Gram-positive bacteria of *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923 strains were used in the study. Before each experiment, bacterial strains were inoculated on 5% sheep blood agar (BESLAB, Turkey) and incubated at 37 °C for 18-20 hours.

Preparation of Curcumin and Gentamicin Sulfate Stock Solutions

Curcumin (Sigma-Aldrich, USA) was weighed 0.00154 g at a concentration of 1000 µg/mL, dissolved in 200 µL 0.5 M sodium hydroxide and 800 µL phosphate buffered saline, filtered and diluted with Mueller Hinton Broth (Himedia, India). Gentamicin sulfate (Biological Industries, Israel) was diluted 1:5000 at a stock concentration of 10 µg/mL and dilutions were prepared.

Determination of Antibacterial Activity

Minimal Inhibitory Concentration Test

Minimal inhibitory concentration (MIC) values for each bacterial strain were performed by liquid microdilution method according to EUCAST recommendations[14]. Serial dilutions of curcumin (1000-1.95 µg/mL) and gentamicin sulfate (10-0.019 µg/mL) were performed in 96-well U-bottom microplates. Bacterial suspensions were diluted to $5x10^5$ CFU/mL and 10 µL each was added to the wells. Positive (growth) and negative (sterility) control wells were also prepared in microplates. Microplates were incubated at 35 ± 1 °C for 18-20 hours. The results were evaluated visually and by spectrophotometer reading. The lowest concentration without growth was considered as the MIC value. Each test was repeated three times.

Minimum Bactericidal Concentration Test

Bacterial concentrations where no growth was observed at the end of incubation were evaluated as minimum bactericidal concentration (MBC) value. In the minimum bactericidal concentration test study, firstly, the wells for which the MIC test was completed and no growth was observed were identified. 2 µL of Mueller Hinton Agar (Merck, Germany) was inoculated from the non-growing wells. The medium plates were incubated at 35±1 °C for 18-20 hours. Bacterial concentrations at the end of incubation were considered as MBC values.

Disc Diffusion Test

Antibacterial activities of curcumin and gentamicin sulfate were determined using disk diffusion test[15]. 0.5 McFarland bacterial suspensions were inoculated on Mueller Hinton Agar. Dilutions of curcumin at concentration ranges of 32-2 mg/mL and gentamicin sulfate at different concentrations selected according to the results of the MIC test were impregnated on 6 mm blank disks (Bioanalyse, Turkey) in 100 µL. Gentamicin (10 µg/mL, Bioanalyse, Turkey) disk was used as a positive control. Petri dishes were then incubated at 35±1 °C for 18-20 hours. Inhibition zone diameters (mm) were measured at the end of incubation. Each test was repeated three times.

Checkerboard Test

The synergistic effect between curcumin and gentamicin sulfate was demonstrated with the checkerboard test[16]. Concentrations were determined at four times above (x16 MIC) and three times below (1/8 MIC) the MIC value. Serial dilutions were prepared with curcumin on the vertical axis and gentamicin sulfate on the horizontal axis. In 96-well U-bottom microplates, 100 µL combinations of curcumin (50 µL) and gentamicin sulfate (50 µL) were prepared in each well. The prepared 0.5 McFarland bacterial suspensions were diluted 1:10 and 5 µL was added to each well to ensure a final bacterial concentration of $5x10^5$ CFU/mL. Wells for positive (growth) and negative (sterility) controls were also prepared in microplates. Microplates were incubated at 35±1 °C for 18-20 hours. The results were evaluated visually and spectrophotometrically at 490-630 nm.

The synergy relationship between curcumin and gentamicin sulfate was calculated as fractional inhibitor concentrations (FIC) sums and fractional inhibitor concentration index (FICI) as follows.

$$FIC_{CURCUMIN} = \frac{\text{MIC of curcumin in combination}}{\text{MIC of curcumin alone}}$$

$$FIC_{GENTAMICIN\ SULFATE} = \frac{\text{MIC of gentamicin sulfate in combination}}{\text{MIC of gentamicin sulfate alone}}$$

$$FICI = \frac{\text{MIC of curcumin in combination}}{\text{MIC of curcumin alone}} + \frac{\text{MIC of gentamicin sulfate in combination}}{\text{MIC of gentamicin sulfate alone}}$$

Synergy between curcumin and gentamicin sulfate was evaluated by calculating the FICI. If FICI ≤0.5, it was interpreted as synergistic effect; if 0.5 < FICI ≤1, it was interpreted as additive effect; if 1 < FICI ≤4, it was interpreted as differential effect; and if FICI >4, it was interpreted as antagonistic effect[16].

Statistical Analysis

Statistical analysis of the data obtained for the disk diffusion test applied in the study was performed with the GraphPad Prism 8 program. Mean±standard deviation values of inhibition zone diameters were calculated in these experiments performed in triplicate.

RESULTS

Antibacterial Activity Results

In the MIC test, the antibacterial activities of curcumin (1000-1.95 µg/mL) and gentamicin sulfate (10-0.019 µg/mL) were tested on standard reference strains. The results of the MIC and MBC tests are shown in Table 1. It was determined that curcumin was effective in all bacteria tested and generally showed activity at a concentration of 62.5 µg/mL. The antibacterial activity of curcumin was highest in the Gram-positive bacteria

Table 1. MIC and MBC values of curcumin and gentamicin sulfate

Bacteria strains	Curcumin		Gentamicin sulfate	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
P. aeruginosa	62.5	125	0.16	0.31
K. pneumoniae	62.5	125	0.08	0.08
P. vulgaris	62.5	62.5	0.16	0.16
B. cereus	62.5	125	0.31	0.31
E. faecalis	7.81	31.25	2.5	5
L. monocytogenes	62.5	62.5	0.31	0.31
S. aureus	62.5	62.5	0.31	0.31

MIC: Minimal inhibitory concentration, MBC: Minimum bactericidal concentration

E. faecalis (7.81 µg/mL). For gentamicin sulfate, antibacterial activity was found at different concentrations in the tested bacteria. MIC and MBC results were obtained at a concentration of 0.31 µg/mL in three bacteria (*B. cereus*, *L. monocytogenes*, *S. aureus*). The most sensitive bacteria to gentamicin sulfate was *K. pneumoniae*, while the most resistant bacteria was *E. faecalis*. At the same time, it was determined that the MIC and MBC results of gentamicin sulfate were compatible (except *E. faecalis*).

Disk diffusion test showed that curcumin showed antibacterial activity at concentrations of 32 and 16 mg/mL. Similar zone diameters were obtained at both concentrations. The most sensitive bacteria to curcumin were *K. pneumoniae*, *E. faecalis* and *L. monocytogenes*, which formed inhibition zone diameter at 4 mg/mL concentration (Table 2).

As a result of the disk diffusion test, the most sensitive bacteria to gentamicin sulfate were *P. aeruginosa*, *B. cereus*, *L. monocytogenes* and *S. aureus*. The lowest concentration effective against these bacteria was 0.63 µg/mL. The most resistant bacteria to gentamicin sulfate was *E. faecalis* (Table 3).

Checkerboard Test Results

Checkerboard test was used to evaluate the synergistic effect between curcumin and gentamicin sulfate. The MIC values of gentamicin sulfate alone and in combination with curcumin are given in Table 4. It was observed that the combination of curcumin and gentamicin sulfate decreased the MIC values in *P. vulgaris*, *B. cereus*, *L. monocytogenes* and *S. aureus* bacteria (Table 4).

The FIC and FICI values of the bacterial strains tested in the study are given in Table 5. Among the Gram-negative bacteria tested, 1 showed additive effect and 2 showed differential effect, while 3 gram-positive bacteria showed additive effect and 1 showed differential effect. The lowest FICI value (0.75)

Table 2. Inhibition zone diameters formed at curcumin concentrations (mm)

Bacteria strains	Inhibition zones (mm) (mean±standard deviation)				
	Curcumin				Gentamicin
	32 mg/mL	16 mg/mL	8 mg/mL	4 mg/mL	10 µg/mL
P. aeruginosa	7.17±0.08	7.11±0.08	7.02±0.08	NZ	21.17±0.07
K. pneumoniae	8.15±0.05	7.13±0.05	7.06±0.06	7.02±0.01	23.02±0.11
P. vulgaris	9.83±0.10	8.84±0.04	8.65±0.05	NZ	23.02±0.13
B. cereus	8.11±0.06	8.04±0.08	NZ	NZ	22.02±0.09
E. faecalis	9.13±0.03	8.14±0.06	8.11±0.04	7.07±0.04	20.06±0.14
L. monocytogenes	9.12±0.12	8.07±0.09	8.03±0.03	7.05±0.03	21.12±0.12
S. aureus	8.16±0.07	8.02±0.06	NZ	NZ	23.12±0.11

NZ: No zone

Table 3. Inhibition zone diameters formed at gentamicin sulfate concentrations (mm)

Bacteria strains	Inhibition zones (mm) (mean±standard deviation)				
	Gentamicin sulfate concentrations				Gentamicin
P. aeruginosa	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**0.16 µg/mL**	**10 µg/mL**
	10.04±0.12	7.25±0.14	NZ	NZ	23.01±0.11
K. pneumoniae	**0.63 µg/mL**	**0.31 µg/mL**	**0.16 µg/mL**	**0.08 µg/mL**	**10 µg/mL**
	7.14±0.14	NZ	NZ	NZ	22.04±0.10
P. vulgaris	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**0.16 µg/mL**	**10 µg/mL**
	9.12±0.12	NZ	NZ	NZ	22.16±0.17
B. cereus	**2.5 µg/mL**	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**10 µg/mL**
	14.11±0.10	11.04±0.14	8.16±0.15	NZ	22.03±0.13
E. faecalis	**20 µg/mL**	**10 µg/mL**	**5 µg/mL**	**2.5 µg/mL**	**10 µg/mL**
	17.12±0.12	13.21±0.19	12.01±0.13	NZ	20.12±0.11
L. monocytogenes	**2.5 µg/mL**	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**10 µg/mL**
	14.02±0.12	11.14±0.14	7.28±0.12	NZ	22.91±0.10
S. aureus	**2.5 µg/mL**	**1.25 µg/mL**	**0.63 µg/mL**	**0.31 µg/mL**	**10 µg/mL**
	15.01±0.11	12.53±0.13	8.21±0.10	NZ	23.17±0.06

NZ: No zone

Table 4. MIC values of gentamicin sulfate alone and in combination with curcumin

Bacteria strains	Gentamicin sulfate	
	MIC value alone (µg/mL)	MIC value in combination (µg/mL)
P. aeruginosa	0.16	0.16
K. pneumoniae	0.08	0.16
P. vulgaris	0.16	0.08
B. cereus	0.31	0.08
E. faecalis	2.50	5
L. monocytogenes	0.31	0.16
S. aureus	0.31	0.16

MIC: Minimal inhibitory concentration

Table 5. FIC and FICI values as a result of checkerboard test

Bacteria strains	$FIC_{CURCUMIN}$	$FIC_{GENTAMICIN\ SULFATE}$	FICI	Interaction
P. aeruginosa	0.02	1	1.02	Indifference effect
K. pneumoniae	0.13	2	2.13	Indifference effect
P. vulgaris	0.50	0.50	1	Additive effect
B. cereus	0.50	0.25	0.75	Additive effect
E. faecalis	0.03	2	2.03	Indifference effect
L. monocytogenes	0.50	0.50	1	Additive effect
S. aureus	0.50	0.50	1	Additive effect

FIC: Fractional inhibitor concentrations, FICI: Fractional inhibitor concentration index

in *B. cereus*, a Gram-positive bacterium, suggests that the combination of curcumin and gentamicin sulfate has a partial synergistic effect on this bacterium.

DISCUSSION

The most common treatment for bacterial infections is the use of antibiotics[17]. However, due to the increase in antibiotic resistance in recent years, there is a need to discover new therapeutic agents with strong antibacterial activity[11]. Curcumin is a natural agent whose therapeutic effects have been investigated due to its various biological and medicinal properties[18]. In this study investigating the antibacterial activity of curcumin on some standard strains, it was found that curcumin showed antibacterial activity at a concentration of 62.5 µg/mL against all tested bacteria except *E. faecalis* (7.81 µg/mL). The results of this study and similar studies also emphasize the antibacterial activity of curcumin[19,20]. In a study by Ungphaiboon et al. (2005)[19], in which they investigated the antibacterial activity of curcumin extracts obtained from *Curcuma longa* L. rhizomes on various microorganisms, they found the MIC values of curcumin as 16 and 128 µg/mL for *Bacillus subtilis* NCTC 10073 and *S. aureus* ATCC 25923, respectively. On the other hand, they could not detect any activity against *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Anbari et al. (2021)[20] examined the antibacterial effects of curcumin nanoparticles and pure curcumin on S. *aureus* PTCC 1764. They found that the MIC values of curcumin nanoparticles were in the range of 0.48-0.34 mg/mL, while pure curcumin was 0.56 mg/mL. Compared to pure curcumin, they found that the MIC values of curcumin nanoparticles were lower than pure curcumin due to their smaller size and easier penetration into bacteria. In the studies, it is seen that the MIC values differ in each study due to the preparation of curcumin by different methods, the use of different methods for the determination of antibacterial activity and testing against different microorganism species.

It is known that gentamicin sulfate, an aminoglycoside group, is an agent that shows broad antibacterial activity with low MIC values against Gram-positive and Gram-negative bacteria[21]. In this study, although the MIC values of gentamicin sulfate varied in the bacteria studied, the most resistant bacteria was *E. faecalis* (2.5 µg/mL) and the most sensitive bacteria was *K. pneumoniae* (0.08 µg/mL). At the same time, the MBC results were similar to the MIC results (except for *E. faecalis*). In a similar study, Arisoy et al. (2013)[22] found that the highest gentamicin sulfate MIC values were found in ESBL-producing *Escherichia coli* (1024 µg/mL) and *K. pneumoniae* (256 µg/mL). Dorati et al.[23] (2018) examined the antibacterial activity of gentamicin sulfate and gentamicin sulfate-loaded nanoparticles in clinical isolates. In the study,

they determined the MIC and MBC test results of gentamicin sulfate as 2/4 µg/mL for *Escherichia coli*, 1/2 µg/mL for *P. aeruginosa*, 4/8 µg/mL for *Proteus mirabilis*, 1/1 µg/mL and 8/16 µg/mL for two S. *aureus* isolates. For the reference strain *Escherichia coli* ATCC 25922, the MIC/MBK values were 0.5/0.5 µg/mL. As a result of the study, they concluded that there was no difference between the MIC/MBK test results of gentamicin sulfate and gentamicin sulfate loaded nanoparticles.

Disk diffusion test is another method used to investigate antibacterial activity. In the literature, there are limited number of studies investigating the antibacterial activity of curcumin by disk diffusion method[6,24]. In this study, disk diffusion test was applied in addition to MIC test. The first two highest concentrations of curcumin, 32 and 16 mg/mL, were found to produce zone diameters of inhibition in all bacteria tested, while large zone diameters were observed at low concentrations of gentamicin sulfate. The limited mobility of bacteria in the disk diffusion test compared to liquid culture in the MIC test may cause the results of these two tests to be different[25]. Khan et al. (2021)[6] investigated the antimicrobial activities of curcumin nanoparticles and its aqueous extract (5, 10, 20 mg/mL) by disk diffusion method and used *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC BAA-1705, *P. aeruginosa* ATCC 27853, *S. aureus A*TCC 23235 and *Aspergillus niger* FCBP-PTF0198 strains. They found that the most effective concentration of curcumin nanoparticles and aqueous extract was 20 mg/mL and at this concentration, nanoparticle inhibition zone diameters were higher than aqueous curcumin.

It is thought that synergistic studies will play an important role in the future of antibiotic therapies to overcome antibacterial resistance and prevent new resistance mechanisms[5]. In recent years, the combined use of natural products and antibiotics in the treatment of infectious diseases has come to the fore in order to expand the pool of therapeutic agents and manage emerging resistance. It has been reported that the combination of curcumin, a natural product, with antibiotics increases the membrane permeability of the bacterial cell and facilitates the entry of antibiotics into the cell[18]. In this study, the combination of curcumin with gentamicin sulfate decreased the MIC values in *P. vulgaris, B. cereus, L. monocytogenes* and *S. aureus* bacteria. In addition, the lowest FICI result (FICI=0.75) in *B. cereus* was interpreted as a partial synergistic effect of curcumin and gentamicin sulfate. In similar studies, it has been shown that combinations of curcumin with different antibiotics are antibacterial and these combinations can be used for therapeutic purposes[13,26]. Bahari et al. (2017)[13] investigated the synergistic activity of curcumin with azithromycin and gentamicin against *P. aeruginosa* (PAO1) by checkerboard test and found that the MIC values of the antibiotics alone were higher than the MIC values of the combination with curcumin. As a result of the study, they found that there was a synergistic effect between curcumin and azithromycin (FICI=0.25) and gentamicin (FICI=0.37). Mun et al. (2013)[26] showed that the combination of curcumin with oxacillin, ampicillin, ciprofloxacin and norfloxacin against eight clinical and two reference MRSA strains reduced the MIC values of these antibiotics and showed synergy/partial synergy between them and curcumin. In studies with curcumin, it is seen that the synergistic effect results vary according to the strain and antimicrobial agent used.

This study shows that curcumin alone or in combination with gentamicin sulfate has antimicrobial activity. However, these results need to be supported by large-scale *in vitro* and *in vivo* studies including clinical isolates to clearly determine the effect of curcumin and gentamicin sulfate synergy in the treatment process of bacterial infections.

Study Limitations

The limitation of this study is that the synergistic effects of curcumin and gentamicin sulfate on bacteria isolated from clinical samples were not investigated.

CONCLUSION

In conclusion, the use of different natural therapeutic agents in combination with antibiotics in the empirical treatment of bacterial infections seems promising in terms of reducing the toxicity of antibiotics and increasing their antimicrobial efficacy.

Ethics

Ethics Committee Approval: This study was reviewed and approved by Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee (approval no: 2022.68.04.18, date: 26.07.2022).

Informed Consent: It is a laboratory study that does not require patient consent.

Authorship Contributions:

Concept: B.B., B.E., Design: B.B., B.E., Data Collection or Processing: B.B., B.E., Analysis or Interpretation: B.B., B.E., Literature Search: B.B., B.E., Writing: B.B., B.E.

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