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# *In-vitro* antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils

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**Abstract**

Spices and herbs have been used for many years by different cultures. The aim of the present study is (1) to investigate *in-vitro* antimicrobial effects of different spices and herbs (5 species: *Rosmarinus officinalis* (Rosemary), *Coriandrum sativum* (coriander), *Micromeria fruticosa* (L.) Druce subsp. Brachycalyx P.H. Davis (White micromeria), *Cumium cyminum* (cumin), *Mentha piperita* (Peppermint) against different bacteria and fungi species, and (2) to discuss the *in-vitro* possible effects between the plants and antibiotics. The microorganisms used were *Micrococcus luteus* LA 2971, *Bacillus megaterium* NRS, Bacillus brevis FMC 3, *Enterococcus faecalis* ATCC 15753, *Pseudomonas pyocyaneus* DC 127, *Mycobacterium smegmatis* CCM 2067, *Escherichia coli* DM, *Aeromonas hydrophila* ATCC 7966, *Yersinia enterocolitica* AU 19, *Staphylococcus aureus* Cowan 1, *Streptococcus faecalis* DC 74 bacteria, and *Saccharomyces cerevisiae* WET 136, *Kluvyeromyces fragilis* DC 98 fungi in this study. The results indicated that essential oils of *Rosmarinus officinalis*, *Coriandrum sativum* L., *Micromeria fruticosa* (L.) Druce subsp. brachycalyx P.H. Davis, *Cumium cyminum* L., *Mentha piperita* L. were shown antimicrobial activity in the range of 7-60 mm 2 $\mu l^{-1}$ inhibition zone to the microorganisms tested, using disc diffusion method. Standard antibiotic such as Gentamicin (10 µg), Cephalothin (30 µg), Ceftriaxone (10 µg), Nystatin (10 U) discs were used for comparison with the antimicrobial activities of essential oils of these plants. In addition, antibacterial activity of essential oils of these plants was researched by effects when it was used together with these standard antibiotics in vitro. However, antibacterial activity changed also by *in vitro* interactions between these standard antibiotics and essential oils of these plants. Synergic, additive or antagonist effects were observed in antibacterial activity.

**Key words**

Spice, Essential oils, Antibacterial and antifungal effects, Synergisms, Additive, Antagonism

## Introduction

Spices and herbs have been used for many years by different cultures to enhance the flavor and aroma of foods, in preserving foods and for their medicinal value. These attributes are useful in the development of snack foods and meat products (Shelef, 1983; Giese, 1994). Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Shelef, 1983; Zaika, 1988; Alzoreky and Nakahara, 2003; Kumral and Sahin, 2003; Park *et al*., 2009).

According to World Health Organization (Santos *et al*., 1995) medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998; Nascimento *et al*., 2000; Mothana *et al*., 2009). Nascimento *et al* (2000) determined that association of antibiotics and plant extracts showed synergistic antibacterial activity against antibiotic-resistant bacteria.

The focal point of Abascal and Yarnell (2002) study is on the combining of herbs with antibiotics to reduce drug resistance acting synergistically with drugs to kill microbes.

The biological activity of the oils can be compared with the activity of synthetically produced pharmacological preparations and should be investigated in the same way. Generally, their action is the result of the combined effect of both active and inactive compounds (Carson and Riley, 2001). These inactive compounds might influence resorption, rate of reactions and bioavailability of the active compounds (Svoboda and Deans, 1995). Several active components might have a synergistic effect (Svoboda and Hampson, 1999). Inactive compounds might serve as growth substrate to microorganisms (Peeiulyte, 2004).

Spices and essential oils can also help and give us some support and protection. The most important suggested areas of essential oil use are in urology, dermatology, sleep and nervous disorders, laxatives, erosive gastritis, cardiac and vascular system, immunomodulating drugs, colds and coughs (Bown, 1995; Baytop, 1999; Svoboda and Hampson, 1999). Spices and essential oils have gained great importance due to their antimicrobial properties. Some authors showed the antimicrobial activity of several spices and essential oils (Kivanc and Akgul, 1986; Kivanc and Akgul, 1988; Deans and Svoboda, 1988; Akgul and Kivanc, 1989; Erdogrul, 2002; De *et al*., 2003; Dulger and Gonuz, 2004). Essential oils represent very complex mixtures of compounds, varied from monoterpenes to sesquiterpenes (Simon *et al*., 1984; Bown, 1995; EMEA, 1998; Svoboda and Hampson, 1999, Oumzil *et al.,* 2002, Esiyok *et al*., 2004; Gulluce *et al*., 2004, Li and Jiang, 2004).

The objective of this research was to evaluate the antibacterial and antifungal activity of essential oils of some spices and herbs against standard microorganism strains. Further, the combined effect of essential oils and antibiotics was also assessed.

## Materials and Methods

The plants used in this study were *Rosmarinus officinalis*, *Coriandrum sativum* L., *Micromeria fruticosa* (L.) Druce ssp. brachycalyx P.H. Davis, *Cumium cyminum* L., *Mentha piperita* L. Plants were dried, broken into pieces using blender and extracted for essential oil as for the method described in European Pharmacopoeia (1975).

The tested microorganisms were provided from the culture collections of the Microbiology Laboratory of the Science and Art Faculty of the University of Kahramanmaras Sutcu Imam, in Kahramanmaras Turkey. These include *Micrococcus luteus* LA 2971, *Bacillus megaterium* NRS, *Bacillus brevis* FMC 3, *Enterococcus faecalis* ATCC 15753, *Pseudomonas pyocyaneus* DC 127, *Mycobacterium smegmatis* CCM 2067, *Escherichia coli* DM, *Aeromonas hydrophila* ATCC 7966, *Yersinia enterocolitica* AU 19, *Staphylococcus aureus* Cowan 1, *Streptococcus faecalis* DC 74 bacteria, and *Saccharomyces cerevisiae* WET 136, *Kluvyeromyces fragilis* DC 98 fungi. Gentamicin (10 µg) (Bioanalyse), Cephalothin (30 µg), Ceftriaxone (10 µg) and Nystatin (100 U) (Oxoid) discs were used as standard antibiotics.

The essential oils obtained were individually injected into empty sterilized antibiotic discs of 6 mm diameter (Schleicher and Shull No: 2668, Germany). In addition Standard antibiotic discs were also used. 2 µl of each essential oil was saturated to antibiotic discs for determination of inhibition zones (Brooks *et al*., 1995; Toroglu, 2007).

The bacteria were incubated in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24 hr, and the yeasts in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1°C for 24 hr. The bacteria and yeasts were injected into petri dishes (9 cm) in the amount of 0.01 ml ($10^6$ $ml^{-1}$ for the bacteria and $10^5$ $ml^{-1}$ for the fungi) (NCCLS, 2000), 15 ml of Mueller Hinton Agar (MHA, Oxoid) and Sabouraud Dextrose Agar (SDA) (sterilized in a flask and cooled to 45-50°C) were homogenously distributed onto the sterilized petri dishes (Collins *et al*., 1989; Bradshaw, 1992).

Sterilized blank paper discs 6 mm in diameter were saturated with 2 ml of essential oil by microinjector (Hamilton) per disc. Standard antibiotic discs were unsaturated and saturated with 2 ml of essential oil per disc then placed onto the agar plates which had previously been inoculated with the above organisms. The petri dishes were left at 4°C for 2 hr, and then the injected plates with bacteria were incubated at 37±0.1°C for 24 hr, plates inoculated with fungi were incubated at 25±0.1°C for 48 hr (Collins *et al*., 1989; Bradshaw, 1992). At the end of the period, the diameter of inhibition zones were measured in mm. These studies were performed in triplicate.

## Results and Discussion

*R. officinalis* essential oil had antimicrobial effects on and among all tested bacteria and fungi. While the inhibition zone was highest in *P. pyocyaneus,* it was lowest in *E. faecalis*. Among the fungi, the inhibition zone against *K. fragilis* was higher than *S. cerevisiae* (Table 1). An antagonistic effect was seen in all the tested bacteria when the combination of *R. Officinalis* essential oil and Gentamicin antibiotic discs were applied. On the other hand, while a synergic effect was seen in *S. aureus* in Celaphalotin antibiotic disc when the combination of *R. officinalis* essential oil and Cephalotin and Ceftriaxone antibiotic discs were applied, additive effect occured in Ceftriaxone antibiotic disc. An antagonistic effect was seen in the other bacteria that were studied (Table 1). While there was no effect of *R. officinalis* with Ceftriaxone antibiotics against *S. aureus*, its application with Cephalothin antibiotics led to higher antimicrobial activity. The application of *R. officinalis* with Gentamicin, Cephalothin and Ceftriaxone led to decrease the antimicrobial activity against other tested bacteria.

Previous works have reported the antimicrobial properties of phenolic diterpenoids of extract of Labiatae (Moujir *et al*., 1993; Del Campo *et al*., 1998; EMEA, 1998). Other authors (Panizzi *et al*., 1993; Del Campo *et al.,* 1998; Mangena and Muyima, 1999; Erdogrul, 2002) also showed the antimicrobial activity of various extracts and essential oil of *Rosmarinus officinalis* on different bacteria and fungi. In another study, althought the ethanolic extract of *Rosmarinus officinalis* showed antibacterial effect against *S. aureus*, *P.aeruginosa*, *B. cereus*, *M. smegmatis*, *M. luteus,* it showed no antifungal effect against *C. albicans*, *K. fragilis*, *R. rubra* (Dulger and Gonuz, 2004). This study was not confirmed our results. Because, in our study, essential oil of *R. officinalis* showed antifungal effect against *S. cerevisiae*, *K. fragilis*. According to other authors (Caceres *et al*., 1987 and Erdogrul, 2002), the ethanol extracts of *R. officinalis* showed an inhibitory effect against *C. albicans*. Our results were similar with this study, despite the different extraction method used and different fungi used. It is thought that observed differences may the result of the combined effect of both active and inactive compounds of plants in this study. In addition, these

**Table - 1:** Antimicrobial activities of *Rosmarinus officinalis* essential oil and its synergistic effect on antibiotics

| Microorganisms | Inhibition zones (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | | | | C | | |
| | 2 µl | G | C | F | N | G | C | F |
| *Escherichia coli* | 8 | 25 | 25 | 38 | nt | 25 | 20 | 37 |
| *Micrococcus luteus* | 12 | 26 | 16 | 8 | nt | 31 | 21 | 14 |
| *Staphylococcus aureus* | 8 | 30 | 17 | 9 | nt | 34 | 26 | 17 |
| *Mycobacterium smegmatis* | 8 | 25 | 18 | 10 | nt | 29 | 22 | 16 |
| *Pseudomonas pyocyaneus* | 14 | 26 | 16 | 9 | nt | 36 | 22 | 17 |
| *Yersinia enterolitica* | 8 | 25 | 14 | 9 | nt | 28 | 18 | 14 |
| *Aeoromonas hydrophila* | 8 | 25 | 18 | 11 | nt | 30 | 18 | 17 |
| *Enterococcus faecalis* | 7 | 24 | 36 | 20 | nt | 27 | 18 | 19 |
| *Bacillus megaterium* | 8 | 24 | 15 | 9 | nt | 31 | 20 | 16 |
| *Streptococcus faecalis* | 10 | 25 | 17 | 10 | nt | 31 | 20 | 16 |
| *Bacillus brevis* | 8 | 23 | 15 | 10 | nt | 28 | 21 | 16 |
| *Saccharomyces cerevisiae* | 10 | nt | nt | nt | 18 | nt | nt | nt |
| *Klyveromyces fragilis* | 14 | nt | nt | nt | 18 | nt | nt | nt |

A: Inhibition zones that occured of plant essential oil,
B: Inhibition zones that occured of standard antibiotic disc,
C: Inhibition zones that occured when essential oil and standard antibiotic are used together,
G: Gentamicin, C: Cephalothin, F: Ceftriaxone, N: Nystatin, nt: Not tested

**Table - 2:** Antimicrobial activities of *Coriandrum sativum* L. essential oil and its synergistic effect on antibiotics

| Microorganisms | Inhibition zones (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | | | | C | | |
| | 2 µl | G | C | F | N | G | C | F |
| *Escherichia coli* | 8 | 25 | 25 | 38 | nt | 21 | 21 | 30 |
| *Micrococcus luteus* | - | 26 | 16 | 8 | nt | 20 | 8 | 7 |
| *Staphylococcus aureus* | - | 30 | 17 | 9 | nt | 30 | 14 | 10 |
| *Mycobacterium smegmatis* | - | 25 | 18 | 10 | nt | 24 | 17 | 11 |
| *Pseudomonas pyocyaneus* | 16 | 26 | 16 | 9 | nt | 24 | 14 | 7 |
| *Yersinia enterolitica* | 8 | 25 | 14 | 9 | nt | 23 | 14 | 8 |
| *Aeoromonas hydrophila* | - | 25 | 18 | 11 | nt | 20 | 14 | 9 |
| *Enterococcus faecalis* | - | 24 | 36 | 20 | nt | 22 | 12 | 8 |
| *Bacillus megaterium* | 8 | 24 | 15 | 9 | nt | 20 | 11 | 7 |
| *Streptococcus faecalis* | 10 | 25 | 17 | 10 | nt | 23 | 13 | 12 |
| *Bacillus brevis* | - | 23 | 15 | 10 | nt | 22 | 12 | 8 |
| *Saccharomyces cerevisiae* | 7 | nt | nt | nt | 18 | nt | nt | nt |
| *Klyveromyces fragilis* | 7 | nt | nt | nt | 18 | nt | nt | nt |

A: Inhibition zones that occured of plant essential oil,
B: Inhibition zones that occured of standard antibiotic disc,
C: Inhibition zones that occured when essential oil and standard antibiotic are used together,
G: Gentamicin, C: Cephalothin, F: Ceftriaxone, N: Nystatin, nt: Not tested

differences among bacteria species may the result of the cell wall in gram-positive bacteria of a single layer, whereas the gram-negative cell wall is multi-layered structure and the yeast cell wall is quite complex.

The inhibition zone of *C. sativum* essential oil were seen against *E. coli*, *P. pyocyaneus*, *Y. enterolitica*, *B. megaterium*, *S. faecalis* bacteria, *S. cerevisiae*, *K. fragilis* fungi (7-16 mm / 2 µl) (Table 2). While an additive effect was seen in *S. aureus* when combined application *C. sativum* essential oil (2 µl) and Gentamicin antibiotic discs, an antagonistic effect was seen in the other bacteria. In combined application *C. sativum* essential oil (2 µl) and Cephalothin antibiotic discs was seen an antagonistic effect in all the tested bacteria. A synergic effect was seen in *S. aureus* and *M. smegmatis* when the combination of *C. sativum* essential oil and Ceftriaxone antibiotics discs were applied and antagonistic effect was seen in the other bacteria (Table 2). Application of *C. sativum* with Ceftriaxone antibiotics led to

**Table - 3:** Antimicrobial activities of *Micromeria fruticosa* (L.) Druce ssp. brachycalyx P.H. Davis essential oil and its synergistic effect on antibiotics

| Microorganisms | Inhibition zones (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | | | | C | | |
| | 2 µl | G | C | F | N | G | C | F |
| *Escherichia coli* | 8 | 25 | 25 | 38 | nt | 31 | 28 | 42 |
| *Micrococcus luteus* | 10 | 26 | 16 | 8 | nt | 39 | 21 | 19 |
| *Staphylococcus aureus* | 7 | 30 | 17 | 9 | nt | 36 | 40 | 25 |
| *Mycobacterium smegmatis* | 8 | 25 | 18 | 10 | nt | 34 | 21 | 18 |
| *Pseudomonas pyocyaneus* | 14 | 26 | 16 | 9 | nt | 32 | 18 | 16 |
| *Yersinia enterolitica* | 8 | 25 | 14 | 9 | nt | 36 | 21 | 20 |
| *Aeoromonas hydrophila* | 8 | 25 | 18 | 11 | nt | 33 | 27 | 24 |
| *Enterococcus faecalis* | 7 | 24 | 36 | 20 | nt | 33 | 41 | 16 |
| *Bacillus megaterium* | 8 | 24 | 15 | 9 | nt | 31 | 17 | 16 |
| *Streptococcus faecalis* | 10 | 25 | 17 | 10 | nt | 30 | 16 | 15 |
| *Bacillus brevis* | 8 | 23 | 15 | 10 | nt | 35 | 16 | 14 |
| *Saccharomyces cerevisiae* | 10 | nt | nt | nt | 18 | nt | nt | nt |
| *Klyveromyces fragilis* | 16 | nt | nt | nt | 18 | nt | nt | nt |

A: Inhibition zones that occured of plant essential oil,
B: Inhibition zones that occured of standard antibiotic disc,
C: Inhibition zones that occured when essential oil and standard antibiotic are used together,
G: Gentamicin, C: Cephalothin, F: Ceftriaxone, N: Nystatin, nt: Not tested

**Table - 4:** Antimicrobial activities of *Cumium cyminum* L. essential oil and its synergistic effect on antibiotics

| Microorganisms | Inhibition zones (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | | | | C | | |
| | 2 µl | G | C | F | N | G | C | F |
| *Escherichia coli* | 10 | 25 | 25 | 38 | nt | 29 | 23 | 46 |
| *Micrococcus luteus* | 16 | 26 | 16 | 8 | nt | 26 | 15 | 17 |
| *Staphylococcus aureus* | 14 | 30 | 17 | 9 | nt | 34 | 44 | 23 |
| *Mycobacterium smegmatis* | 18 | 25 | 18 | 10 | nt | 18 | 14 | 24 |
| *Pseudomonas pyocyaneus* | 10 | 26 | 16 | 9 | nt | 46 | 35 | 34 |
| *Yersinia enterolitica* | 16 | 25 | 14 | 9 | nt | 35 | 29 | 26 |
| *Aeoromonas hydrophila* | 20 | 25 | 18 | 11 | nt | 50 | 70 | 43 |
| *Enterococcus faecalis* | 46 | 24 | 36 | 20 | nt | 30 | 19 | 42 |
| *Bacillus megaterium* | 16 | 24 | 15 | 9 | nt | 22 | 15 | 13 |
| *Streptococcus faecalis* | 14 | 25 | 17 | 10 | nt | 35 | 16 | 12 |
| *Bacillus brevis* | 14 | 23 | 15 | 10 | nt | 31 | 18 | 19 |
| *Saccharomyces cerevisiae* | 20 | nt | nt | nt | 18 | nt | nt | nt |
| *Klyveromyces fragilis* | 60 | nt | nt | nt | 18 | nt | nt | nt |

A: Inhibition zones that occured of plant essential oil,
B: Inhibition zones that occured of standard antibiotic disc,
C: Inhibition zones that occured when essential oil and standard antibiotic are used together,
G: Gentamicin, C: Cephalothin, F: Ceftriaxone, N: Nystatin, nt: Not tested

increase of antibacterial activity against *S. aureus* and *M. smegmatis*. Application of *C. sativum* with Gentamicin, Cephalothin and Ceftriaxone led to decrease antimicrobial activity against the other bacteria. Lo Cantore *et al*. (2004) and Sing *et al*. (2002) reported that essential oils extracted from *Coriandrum sativum* showed antibacterial activity to different bacteria. These results confirmed our findings.

*Micromeria fruticosa* essential oil showed differed inhibition zones according to the bacteria and fungi. While the inhibition zone was highest in the bacteria *P. pyocyaneus*, it was lowest in *S. aureus* and *E. faecalis*. Among the fungi, the inhibition zone against *K. fragilis* was higher than *S. cerevisiae* (Table 3). A synergic effect was seen in *M. luteus*, *M. smegmatis*, *Y. enterolitica*, *E. faecalis*, *B. brevis* when the combination of *M. fruticosa* essential oil (2 µl) and Gentamicin antibiotic discs were applied and additive effect was seen in *A. hydrophila*. An antagonistic effect was seen in other tested bacteria when the combination of *M. fruticosa* essential oil and Gentamicin antibiotic discs was applied so the inhibition zone decreased. A synergistic effect was seen in *S. aureus* and *A.*

**Table - 5:** Antimicrobial activities of *Mentha piperita* L essential oil and its synergistic effect on antibiotics

| Microorganisms | Inhibition zones (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | | | | C | | |
| | 2 µl | G | C | F | N | G | C | F |
| *Escherichia coli* | 12 | 25 | 25 | 38 | nt | 26 | 22 | 36 |
| *Micrococcus luteus* | - | 26 | 16 | 8 | nt | 29 | 18 | 0 |
| *Staphylococcus aureus* | 24 | 30 | 17 | 9 | nt | 30 | 28 | 18 |
| *Mycobacterium smegmatis* | - | 25 | 18 | 10 | nt | 24 | 18 | 9 |
| *Pseudomonas pyocyaneus* | 10 | 26 | 16 | 9 | nt | 28 | 18 | 10 |
| *Yersinia enterolitica* | 26 | 25 | 14 | 9 | nt | 25 | 21 | 13 |
| *Aeoromonas hydrophila* | 20 | 25 | 18 | 11 | nt | 27 | 19 | 14 |
| *Enterococcus faecalis* | 26 | 24 | 36 | 20 | nt | 24 | 39 | 20 |
| *Bacillus megaterium* | - | 24 | 15 | 9 | nt | 24 | 16 | 0 |
| *Streptococcus faecalis* | - | 25 | 17 | 10 | nt | 25 | 16 | 0 |
| *Bacillus brevis* | - | 23 | 15 | 10 | nt | 22 | 16 | 10 |
| *Saccharomyces cerevisiae* | 14 | nt | nt | nt | 18 | nt | nt | nt |
| *Klyveromyces fragilis* | 14 | nt | nt | nt | 18 | nt | nt | nt |

A: Inhibition zones that occured of plant essential oil,
B: Inhibition zones that occured of standard antibiotic disc,
C: Inhibition zones that occured when essential oil and standard antibiotic are used together,
G: Gentamicin, C: Cephalothin, F: Ceftriaxone, N: Nystatin, nt: Not tested

*hydrophila* when the combination of *M. fruticosa* essential oil and Cephalothin antibiotic discs were applied. Antagonistic effect was seen in other tested bacteria when the combination of *M. fruticosa* essential oil and Cephalothin antibiotic discs was applied so the inhibition zone decreased. In combined application of *M. fruticosa* essential oil and Ceftriaxone antibiotics discs was seen synergistic effect in *M. luteus*, *S. aureus*, *Y. enterolitica*, and *A. hydrophila*, additive effect in *M. smegmatis*, and antagonistic effect in other bacteria (Table 3). Synergic effect was seen in bacteria, the application of *M. fruticosa* with Gentamicin, Cephalothin and Ceftriaxone led to increase the antimicrobial activity.

According to Gulluce *et al.* (2004), essential oil of the *M. fruticosa* ssp. *serpyllifolia* exhibited activity against 14 bacteria, three fungi and a yeast, whilst the methanolic extract was inactive. Also, Shimoni *et al.* (1993) found that essential oil of the *M. fruticosa* exhibited antifungal activity against the soil-borne pathogens and the foliar pathogens. Our results were confirmed this situation.

*Cumium cyminum* essential oil had antimicrobial effects on and among all tested bacteria and fungi. While the inhibition zone was higher in the bacteria *E. faecalis,* it was lowest in *E. coli* and *P. pyocyaneus*. Among the fungi, the inhibition zone against *K. fragilis* was higher than *S. cerevisiae* (Table 4). In combined application of *C. cyminum* essential oil (2 µl) and Gentamicin antibiotics discs was seen synergistic effect in *P. pyocyaneus* and *A. hydrophila*, an antagonistic effect was seen in other bacteria. When the combination of *C. cyminum* essential oil and Cephalothin antibiotics discs were applied, a synergistic effect was seen in *S. aureus*, *P. pyocyaneus* and *A. hydrophila*, and an antagonistic effect was seen in other bacteria. A synergic effect was seen against *P. pyocyaneus*, *Y. enterolitica*, *A. hydrophila* when the combination of *C. cyminum* essential oil and Ceftriaxone antibiotics discs were applied. Additive effect was seen in *S. aureus* and antagonistic effect was seen in other bacteria when the combination of *C. cyminum* essential oil and Ceftriaxone antibiotics discs were applied (Table 4). The application of *C. cyminum* with Gentamicin, Cephalothin and Ceftriaxone led to increase the antimicrobial activity in *P. pyocyaneus* and *A. hydrophila.* The application of *C. cyminum* with Gentamicin, Cephalothin and Ceftriaxone led to decrease antimicrobial activity against the other bacteria that were used. However, it may be used as antifungal agent to *K. fragilis*.

According to De *et al*. (2003) the alcohol extract of cumin was tested on different test organisms. Significant growth inhibition was observed for *Agrobacterium tumefaciens* and *B. subtilis*. *M. luteus*, *E. aerogenes* and *E. coli* did not show sensitivity to concentrations that were lower than 100 mg EPW/ml of the extract. However, the cumin extract could not inhibit the growth of *S. cerevisiae* (De *et al*., 2003). Antibacterial and antifungal activities of *C. cyminum* L. essential oil were not similar with De *et al*. (2003) findings. Because, in our study, *C. cyminum* L. had the broadest spectra of activity against all the test microorganisms.

Other authors also showed the antimicrobial activities of hexane extracts and the volatile components of *Cuminum cyminum* (Agnihotri and Vaidya, 1996), water extracts or juices of *C. cyminum* (Kumral and Sahin, 2003), methanolic extracts of *C. cyminum* on different bacteria in different values. Our results concur with these studies. When the results obtained this study were compared to Bonjar (2004 a,b) studies, it was determined that *C. cyminum* essential oil has higher effect against tested microorganisms.

*M. piperita* essential oil formed an inhibition zone against *E. coli*, *S. aureus*, *P. pyocyaneus*, *Y. enterolitica*, *A. hydrophila*, *E.*

*faecalis*, *S. cerevisiae*, *K. fragilis.* The inhibition zone was higher in *Y. enterolitica* and *E. faecalis,* but, lower in *P. pyocyaneus* (Table 5). A synergistic effect was occured in *M. luteus* when the combination of *M. piperita* essential oil (2 µl) and Gentamicin antibiotic discs were applied, additive effect was occured in *B. megaterium* and *S. faecalis*, antagonistic effect was occured other bacteria. In combined application of *M. piperita* essential oil (2 µl) and Cephalothin antibiotics discs was seen synergistic effect in *M. luteus*, *B. megaterium*, *B. brevis*, additive effect was seen *M. smegmatis*, antagonistic effect was seen in other bacteria. When the combination of *M. piperita* essential oil and Ceftriaxone antibiotics discs were applied, additive effect was occured in *B. brevis*, and an antagonistic effect was seen in other bacteria (Table 5). Synergic effect was seen in bacteria, the application of *M. piperita* with tested antibiotics increased the antimicrobial activity. But, antagonistic effect was seen in bacteria, the application of *M. piperita* with tested antibiotics led to decrease antimicrobial activity.

Results associated with the antimicrobial effects of essential oil of *M. piperita* L. (Peppermint oil) were similar to the previous finding in the literature (Sow *et al*., 1995; Pattnaik *et al*., 1996,1998; Mimica-Dukic *et al*., 2003).

Antimicrobial disc susceptibility tests serve as standard assays for measuring the activity of compounds against pathogenic bacteria. According to Settineri and Krassner's study, two plant-derived proprietary essential oil blends were tested for their antibacterial activity five pathogenic bacteria using disc susceptibility test. This screening study indicates that essential oils may be considered to be used in combination with standard topical and antibiotic therapies (Settineri and Krassner, 2003).

According to Abascal and Yarnell (2002), tea (*Camellia sinensis*) has antimicrobial action against many pathogenic bacteria, including methicillin-resistant *S. aureus*; a synergistic effect when combined with beta-lactam antibiotics (Abascal and Yarnell, 2002). According to Adeleke and Olaitan (2003), application of oil in the dispersion of a water-insoluble antibiotic would be expected to avoid the hydrolytic reaction, thereby, facilitating the antibacterial activity of the compound. Muroi and Kubo (1996) observed that the antimicrobial activity of anacardic acid as phytochemical on different pathogens. Later, it was tested the bactericidal activity of anacardic acid and totarol as phytochemicals on methicillin resistant strains of *S. aureus* (MRSA) and the synergistic effect of these compounds associated with methicillin (Muroi and Kubo, 1996). In the previous studies (Toroglu *et al*., 2005, 2006) that, an antagonistic effect or synergistic effect are seen against whole tested bacteria while different plant essential oils and different standard antibiotics were applied the combination. *In-vitro* effect has found to change when standard antibiotics were used with essential oils. In the later study (Toroglu, 2007) that, an antagonistic effect is seen against whole tested bacteria when the combination of Thymus eigii essential oil and standard antibiotic were applied *In-vitro*.

The essential oils of tested spices and herbs can be used for protection against some bacteria. The focal point this article is suggested that the combination of plants with antibiotics reduced drug resistance. The synergistic effect obtained in few cases could lead to new choices for the treatment of infectious diseases.

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