

## Studies on antimicrobial activities of solvent extracts of different spices

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### Abstract

The antimicrobial activities of the ethyl acetate, acetone and methanol extract of 12 plant species were studied. The extract of *Capsicum annuum* (red pepper) (fruit) *Zingiber officinale* (ginger) (root), *Cuminum cyminum* (cumin), *Alpinia ficinarum* (galingale), *Coriandrum sativum* (coriander), *Cinnamomum zeylanicum* Nees (cinnamomun), *Origanum onites* L. (thyme), *Folium sennae* (senna), *Eugenia caryophyllata* (cloves), *Flos tiliae* (lime), *Folium menthae crispae* (peppermint) and *Piper nigrum* (blackpepper) were tested *in vitro* against 2 fungi and 8 bacterial species by the disc diffusion method. *Klebsiella pneumonia* 13883, *Bacillus megaterium* NRS, *Pseudomonas aeruginosa* ATCC 27859, *Staphylococcus aureus* 6538 P, *Escherichia coli* ATCC 8739, *Enterobacter cloaca* ATCC 13047, *Corynebacterium xerosis* UC 9165, *Streptococcus faecalis* DC 74, *Kluyveromyces marxianus*, *Rhodotorula rubra* were used in this investigation. The results indicated that extracts of different spices has shown antibacterial activity in the range of 7-24 mm  $30\mu\text{l}^{-1}$  inhibition zone *Eugenia caryophyllata* (clove), 7-20 mm  $30\mu\text{l}^{-1}$  inhibition zone *Capsicum annuum* (red pepper) and *Cinnamomum zeylanicum* (cinnamon) bark, 7-18 mm  $30\mu\text{l}^{-1}$  inhibition zone *Folium sennae* (senna) leaves, 7-16 mm  $30\mu\text{l}^{-1}$  inhibition zone *Zingiber officinale* (ginger) root, 7-15 mm  $30\mu\text{l}^{-1}$  inhibition zone *Cuminum cyminum* (cumin) seed, 7-14 mm  $30\mu\text{l}^{-1}$  inhibition zone *Folium menthae crispae* (peppermint), *Origanum onites* (thyme) leaves and *Alpinia ficinarum* (galingale) root, 7-12 mm  $30\mu\text{l}^{-1}$  inhibition zone *Piper nigrum* (blackpepper), 7-11 mm  $30\mu\text{l}^{-1}$  inhibition zone *Flos tiliae* (lime) leaves, 7-8 mm  $30\mu\text{l}^{-1}$  inhibition zone *Coriandrum sativum* (coriander) to the microorganisms tested.

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### Introduction

Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods (Meng *et al.*, 1998). Consequently, there is considerable interest in ways to stop this upward trend and reduce the incidence of food poisoning. Spices and herbs have been added to food since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Nakatani, 1994). Increasing of infections based on antibiotic resistant microorganisms and increasing conscious food consumers have to be using new and natural antimicrobials (Duman–Aydin, 2008). One area of research is the development of new and improved methods of food preservation. Additives are harmful for human health, especially

monosodium glutamate, aspartame, saccharin, sodium cyclamate, sulfites, nitrates, nitrites and antibiotics. It causes headache, nausea, weakness, mental retardation, seizures, cancer and anorexia (Rangan and Barceloux, 2009). As a result of these, consumers interest in natural products, especially plant extracts, including their essential oils and essences. It is well established that these extracts (anise, cumin, dalmagya sage, dill, fennel, laurel, mint, oregano, pickling herb, rosemary, sage, summer savory, sea fennel, sumac and black thyme) have antimicrobial properties against bacteria, moulds and yeasts (Farag *et al.*, 1989; Dorman and Svoboda, 2000; Ozcan and Erkmén, 2001; Sagdic, 2003; Sagdic and Ozcan, 2003). Antimicrobial properties of certain spices have been reported in meat and meat products, e.g. poultry meat, turkey breast and beef, broth and foods and turkey frankfurter slurries (Nkanga and Uraih, 1981; Hall and Maurer, 1986; Akgul and Kivanc, 1989b; Deans and Svoboda, 1990).

The aim of this study was to compare the inhibitory effects of spice extracts on the growth of some bacteria and fungi. In the present study, the antibacterial activities of methanol, ethylene acetate and acetone extracts of 12 plant species were studied *in vitro* against 2 fungi and 8 bacterial species and strains by the disc diffusion method and the results are discussed.

### Materials and Methods

**Spice samples:** *Capsicum annuum* (Red pepper), *Zingiber officinale* (Ginger), *Cuminum cyminum* (Cumin), *Alpinia ficinarum* (Galingale), *Coriandrum sativum* (Coriander), *Cinnamomum zeylanicum* Nees (Cinnamon), *Origanum onites* (Thyme) L., *Folium sennae* (Senna), *Eugenia caryophyllata* (Clove), *Flos tiliae* (Lime), *Folium menthae crispae* (Peppermint) and *Piper nigrum* (Blackpepper) were taken from different spice sellers in the Çine (Aydin) region, Turkey. The taxonomic identities of these spices were authenticated by Dr. Cenet at the Biology Department of KSU's Science and Art Faculty according to the conventional method (Table 1). (Davis, 1965; Baytop, 1999).

**Test of antimicrobial activity:** The spices parts used were dried and broken into small pieces under sterile conditions, and 20 g of each plant tested part (Table 1) were extracted with 150 ml of ethyl acetate, acetone and methanol (Merck, Darmstadt; purity 99.5, 99.8 and 99.9%, respectively) for 24 hr by Soxhlet equipment (Khan *et al.*, 1988; Alzoreky and Nakahara, 2003). Most of the solvents of ethyl acetate, acetone or methanol extracts were evaporated *in vacuo* at 30°C using a rotary evaporator until 1ml.

The disc assay described by Bauer *et al.* (1966) was used. All of the extracts individually were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Schül No:2668, Germany) in the amount of 30µl. Discs injected with pure ethyl acetate, acetone and methanol served as negative controls.

The bacteria were incubated in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24 hr, and the fungi were incubated in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1°C for 24 hr. The bacteria (*Klebsiella pneumonia* 13883, *Bacillus megaterium* NRS, *Pseudomonas aeruginosa* ATCC 27859, *Staphylococcus aureus* 6538 P, *Escherichia coli* ATCC 8739, *Enterobacter cloaca* ATCC 13047, *Corynebacterium xerosis* UC 9165, and *Streptococcus faecalis* DC 74) and fungi (*Kluyveromyces marxianus*, *Rhodotorula rubra*) were injected into petri dishes (9 cm) in the amount of 0.01ml (10<sup>6</sup> ml<sup>-1</sup> for the bacteria and 10<sup>5</sup> ml<sup>-1</sup> for the fungi) (NCCLS, 2000), 15 ml of Muller-Hinton agar (MHA, Oxoid) and Sabouraud Dextrose Agar (SDA) (sterilized in a flask and cooled to 45-50°C) were distribute the medium homogeneously (Collins *et al.*, 1989).

Disk injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4°C for 1-2 hr and then the injected plates with bacteria were incubated at 37±0.1°C for 18-24 hr, plates inoculated with fungi were incubated at 25±0.1°C for 48 hr (Collins *et al.*, 1989; Bradshaw, 1992; Toroglu, 2007). At the end of the period, the inhibition zones

formed on the media were measured with a transparent ruler in millimeters.

### Results and Discussion

The *in vitro* antibacterial activities of the dried extracts of *Capsicum annuum*, *Zingiber officinale*, *Cuminum cyminum*, *Alpinia ficinarum*, *Coriandrum sativum*, *Cinnamomum zeylanicum* Nees, *Origanum onites* L., *Folium sennae*, *Eugenia caryophyllata*, *Flos tiliae*, *Folium menthae crispae* and *Piper nigrum* were shown Table 1.

*Eugenia caryophyllata* (clove) flower and stem extracts various antibacterial activity (7-24 mm 30 µl<sup>-1</sup> inhibition zone) to the microorganisms tested. The methanol extracts did not inhibit *Klebsiella pneumonia* like acetone and ethyl acetate. The acetone extracts showed no inhibition to *R. rubra* and *K. pneumoniae*. The ethyl acetate extracts showed antibacterial activity 7 mm 30µl<sup>-1</sup> only *B. megaterium* NRS. Burt and Reinders (2003) were obtained antimicrobial activity of *E. caryophyllata* like Liu and Nakano (1996) and Agaoglu *et al.* (2006) and Shan *et al.* (2007).

*Capsicum annuum* (redpepper) fruit extracts showed various antibacterial activity (7-20 mm 30µl<sup>-1</sup> inhibition zone) to the microorganisms tested. The methanol extracts did not inhibit microorganisms tested except for *P. aeruginosa*. While the acetone extracts showed antibacterial activity *P. aeruginosa* and *R. rubra*, the ethyl acetate extracts showed antibacterial activity *P. aeruginosa* and *K. pneumoniae* among the listed microorganisms. Erturk (2006) was obtained antimicrobial activity of *C. annuum*. However Agaoglu *et al.* (2006) reported that *C. annuum* showed no antibacterial activity like Sagdic *et al.* (2003) and Liu and Nakano (1996). These findings are not accordance with finding of ours. It can be suggested that different climates plants grown and different extraction methods might be effect the antimicrobial activity.

*Cinnamomum zeylanicum* (cinnamon) bark extracts showed antibacterial activity (7-20 mm 30 µl<sup>-1</sup> inhibition zone) to the microorganisms tested. The methanol extracts showed antibacterial activity against the microorganisms tested. The ethyl acetate extracts showed no inhibition *P. aeruginosa* and *R. rubra*. The acetone extracts showed antibacterial activity the microorganisms tested except for *R. rubra*. Agaoglu *et al.* (2006) were obtained that *C. zeylanicum* the most effective spice against all of the test strains except *M. luteus*. Smith-Palmer *et al.* (1998) were found that the oils of cinnamon were the most inhibitory, each having a bacteriostatic concentration of 0.075% or less against all of five pathogens (*S. aureus*, *L. monocytogenes*, *Camphylobacter jejuni*, *Salmonella enteritidis*, *E. coli*).

*Folium sennae* (senna) leaves extracts various antibacterial activity (7-18 mm 30 µl<sup>-1</sup> inhibition zone) to the microorganisms tested. The methanol extracts showed no inhibition *P. aeruginosa* and *R. rubra* like ethyl acetate extracts. But The acetone extracts showed antibacterial activity to *S. faecalis* and *K. marxianus*. Ali *et al.* (1999) reported that senna showed 100% growth inhibition of *Staphylococcus pyogenes* and *Corynebacterium diptheria*. In a

**Table - 1:** Antimicrobial activity of the plants as mean of inhibition diameter zone (mm) (30 µl disc<sup>-1</sup>)

Plant name local name	Extracts	Inhibition diameter zone (mm)										
		Bacteria								Fungi		Negative controls
		K.p	B.m	P.a	S.a	E.c	E.cl	C.x	S.f	K.m	R.r	
Clove (Plant+Stem) (Karanfil)	Methanol	-	14	12	12	8	10	10	12	24	16	0
	Acetone	-	12	8	10	8	9	8	12	18	-	0
	Ethyl acetate	-	7	-	-	-	-	-	-	-	-	0
Redpepper (Fruit) (Kirmizi biber)	Methanol	-	-	7	-	-	-	-	-	-	-	0
	Acetone	-	-	8	-	-	-	-	-	-	20	0
	Ethyl acetate	-	-	7	-	-	-	-	-	14	-	0
Cinnamon (Bark) (Tarcin)	Methanol	12	14	7	10	14	12	14	12	20	11	0
	Acetone	10	13	7	11	12	12	12	10	16	-	0
	Ethyl acetate	7	7	-	7	8	7	7	10	10	-	0
Senna (Leaves) (Sinnameki)	Methanol	8	8	-	8	8	8	8	8	11	-	0
	Acetone	-	-	-	-	-	-	-	7	18	-	0
	Ethyl acetate	9	7	-	7	9	7	7	10	15	-	0
Zinger (Root) (Zencefil)	Methanol	10	8	7	10	10	10	10	12	16	-	0
	Acetone	-	-	7	-	8	-	-	-	8	-	0
	Ethyl acetate	7	8	-	8	8	7	8	7	10	-	0
Peppermint (Leaves+Flower) (Nane)	Methanol	-	12	-	-	-	-	-	8	12	-	0
	Acetone	-	-	-	-	-	-	-	-	14	-	0
	Ethyl acetate	-	-	-	7	-	7	7	7	11	-	0
Thyme (Leaves+Flower) (Kekik)	Methanol	-	-	-	-	7	7	-	9	-	-	0
	Acetone	-	8	-	7	7	-	-	8	-	-	0
	Ethyl acetate	8	9	-	8	7	7	7	7	14	-	0
Galingale (Root) (Havlican)	Methanol	8	7	8	8	8	8	8	8	14	-	0
	Acetone	-	-	7	-	-	-	-	7	9	-	0
	Ethyl acetate	8	8	7	8	8	8	10	10	13	-	0
Cumin (Fruit) (Kimyon)	Methanol	9	12	7	8	8	8	8	8	11	-	0
	Acetone	8	7	7	7	8	7	7	-	15	-	0
	Ethyl acetate	10	12	8	12	10	8	8	10	11	-	0
Blackpepper (Fruit) (Karabiber)	Methanol	-	7	-	7	8	7	7	8	10	-	0
	Acetone	-	-	-	-	-	-	7	-	-	-	0
	Ethyl acetate	7	8	-	8	8	7	7	-	12	-	0
Lime (Leaves+Flower) (Ihlamur)	Methanol	-	-	7	-	7	7	7	-	10	-	0
	Acetone	-	-	-	-	-	-	-	-	-	-	0
	Ethyl acetate	7	8	7	8	7	7	-	8	11	-	0
Coriander (Seed) (Kisnis)	Methanol	-	-	-	-	-	-	-	-	8	-	0
	Acetone	-	8	-	-	-	-	-	-	-	-	0
	Ethyl acetate	-	7	-	-	7	7	7	-	8	-	0

- Not inhibited, K.p= *Klebsiella pneumoniae* 13883, B.m= *Bacillus megaterium* NRS, P.a= *Pseudomonas aeruginosa* ATCC 27859, S.a= *Staphylococcus aureus* 6538 P, E.c= *Escherichia coli* ATCC 8739, E.cl= *Enterobacter cloaca* ATCC 13047, C.x= *Corynebacterium xerosis* UC 9165, S.f= *Streptococcus faecalis* DC 74, K.m= *Kluyveromyces marxianus*, R.r= *Rhodotorula rubra*

different study, senna did not show activity any of the bacteria tested (Srinivasan *et al.*, 2001).

*Zingiber officinale* (zinger) root extracts showed various antibacterial activities (7-16 mm 30 $\mu$ l<sup>-1</sup> inhibition zone) to the microorganisms tested. The acetone extracts showed antibacterial activity *P. aeruginosa*, *E. coli* and *K. marxianus*. The ethyl acetate extracts showed no inhibition zone to *P. aeruginosa* and *R. rubra*. The methanol extracts showed no inhibition only *R. rubra*. Konning *et al.* (2004) found that the methanol extracts of the plant were significantly active against the bacteria Gram (+) and Gram (-) and fungi studied. The extracts were less active against *P. aeruginosa*, which is naturally resistant to antibacterial agents (Walker and Edwards, 1999). In a similar study, Bonjar *et al.* (2004) the methanol extracts of *Z. officinale* was active against to all of Gram (+) bacteria. These results were in accordance with ours. Different method and essential oil were used by Toroglu *et al.* (2006) and their results similar to ours. But in 2005, Wanissorn *et al.* was obtained that the essential oils of *Z. officinale* were not antibacterial activity. The reason of the different antimicrobial activity was explained by Onyeagba *et al.* (2004) that changes one country to other.

*Cuminum cyminum* (cumin) seed extracts showed 7-15 mm 30  $\mu$ l<sup>-1</sup> inhibition zone against the listed microorganisms. The methanol extracts showed no inhibition against *R. rubra* like ethyl acetate extracts. But the acetone extracts did not inhibit *S. faecalis* and *R. rubra*. In a study an antimicrobial activity of volatile oils of some spices, Con *et al.* (1998) reported that cumin had an inhibitory effect against *S. aureus* and *M. luteus*. In a similar investigation, Akgul and Kivanc, (1989a) reported that cumin exhibited an inhibitory effect against *S. aureus* and *K. pneumoniae* and *P. aeruginosa*. But the results of the present study are different from Con *et al.* (1998) and Akgul and Kivanc (1989a). It can be suggested that the inhibitory effect of cumin might be due to carvone and carvacrol contained in its volatile oil as reported by Ouattara *et al.* (1997).

*Folium menthae crispae* (peppermint) leaves and flower extracts showed antibacterial activity (7-14 mm 30 $\mu$ l<sup>-1</sup> inhibition zone) to the microorganisms tested. The ethyl acetate extracts showed antibacterial activity 7 mm 30 $\mu$ l<sup>-1</sup> inhibition zone to *S. aureus*, *E. cloace*, *C. xerosis*, *S. faecalis*. The methanol extracts showed antibacterial activity *B. megaterium* and *S. faecalis* or *K. marxianus*. The acetone extracts showed no inhibition except for *K. marxianus*. Liu and Nakano (1996) tested the alcoholic extracts of peppermint (%0,1,%0,2 and %0,5) to *E.coli*, *Salmonella*, *Vibrio cholerae*, *S. aureus*. Alcoholic extracts of peppermint (%0,1 and %0,2) showed no inhibition tested bacteria, but %0,5 concentrations showed weak inhibition to *S.aureus*. In a similar study Erturk (2006) showed that peppermint ethanolic extracts showed antimicrobial activity for all the microorganisms tested (*B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*). Freidman *et al.* (2002) reported that peppermint showed antibacterial activity to *Salmonella enterocolitica*. It can be suggested that the inhibitory effect of peppermint might be due to different extracts.

*Alpinia ficinarum* (galingale) root extracts showed various antibacterial activities 7-14 mm 30 $\mu$ l<sup>-1</sup> inhibition zone to the microorganism tested. The methanol extracts showed no inhibition zone only *R. rubra* like ethyl acetate extracts. The acetone extracts showed antibacterial activity against *P. aeruginosa*, *S. faecalis* and *K. marxianus*. Bonjar (2004) reported that methanol extracts of galingale showed no inhibition *K. pneumoniae*, *Bordetella branchiseptica* and *S. aureus*.

*Origanum onites* (thyme) leaves and flower extracts showed antibacterial activities 7-14 mm 30 $\mu$ l<sup>-1</sup> inhibition zone against microorganism tested. The methanol extracts showed no inhibition except for *E. coli* and *E. cloace* or *S. faecalis*. The ethyl acetate showed no inhibition zone to *P. aeruginosa* and *R. rubra*. The acetone extracts showed antibacterial activity against *B. megaterium* NRS, *S. aureus*, *S. faecalis*, *E. coli*. Sarac and Ugur, (2007) were used hydrodistillation method for antimicrobial activities of the essential oils against microorganisms. Essential oil of *O. onites* were effective against to Gram (+) and Gram (-) bacteria. Sagdic, (2003) reported that all tested bacteria were inhibited by hydrosols using paper disc diffusion method. The most sensitive of the bacteria against *O.onites* hydrosols was *S. aureus*. In addition, the most inhibitive of the spice hydrosols on four pathogenic bacteria were *O. onites* like Baratta *et al.* (1998). Baydar *et al.* (2004) showed that the essential oil of *O. onites* at 1/50 concentration was active against all of the bacteria except *Aeromonas hydrophila*, *B. cereus* and *E. coli*. While the oil at 1/200 concentration was only inhibitory effect *Bacillus amyloliquefaciens* and *Proteus vulgaris*, 1/300 concentration of the oil had no inhibitory effect against any of the bacteria. Several researchers reported that *O. onites* inhibited yeasts and bacteria (Kivanc and Akgül, 1986; Sokovic *et al.*, 2002). The reason for the different antimicrobial activity depending on the species, subspecies, or variety. In fact, the essential oils of some plants belonging to the same species that were collected from different locations showed different levels of antimicrobial activities (Sarac and Ugur, 2007).

*Piper nigrum* (black pepper) fruit extracts showed various antibacterial activities (7-12 mm 30 $\mu$ l<sup>-1</sup> inhibition zone) against the microorganisms listed. The acetone extracts showed inhibition zone all microorganism tested except for *C. xerosis*. The methanol extracts showed no inhibition zone to *K. pneumoniae*, *P. aeruginosa* and *R.rubra*. The ethyl acetate extracts showed no inhibition zone *P. aeruginosa* and *S. faecalis* or *R. rubra*. Erturk, (2006) was obtained antimicrobial activity of *P. nigrum*. However Indu *et al.* (2006) found that extracts of *P. nigrum* did not show antibacterial activity against the test microorganisms, the reason of these, plant collection site (India) and bacterial strains (*E. coli*, *Salmonella*, *L. monocytogenes*, *Aeromonas hydrophila*) and extracts (ethanol) different from us.

*Flos tiliae* (lime) leaves and flower extracts various antibacterial activity (7-11 mm 30 $\mu$ l<sup>-1</sup> inhibition zone) to the microorganisms tested. The methanol extracts showed no inhibition against to *K. pneumoniae* and *B. megaterium* or *S. aureus* or *S.*

*faecalis* or *R. rubra*. Surprisingly the acetone extracts showed no inhibition the microorganisms tested. The ethyl acetate extracts showed antibacterial activity the microorganisms tested except for *C. xerosis* and *R. rubra*. Duman-Aydin, (2008) indicated that, the essential oil of lime was decreased 1 log *E. coli* O157:H7, they used lactic, citric, salicylic and sorbic acid extracts but *S. aureus* and *Yersinia enterocolitica* was inactivated by all acid solutions. It can be suggested that the inhibitory effect of lime might be due to different extracts (acid or alcohol).

*Coriandrum sativum* (coriander) seed extracts various antibacterial activity 7-8 mm 30 $\mu$ l<sup>-1</sup> inhibition zone) to the microorganisms tested. The methanol extracts showed antibacterial activity against to only *K. marxianus* and the acetone extracts showed antibacterial activity against only *B. megaterium*. The ethyl acetate extracts showed no inhibition zone to *K. pneumoniae* and *P. aeruginosa* or *S. aureus* or *S. faecalis* or *R. rubra*. Some researchers studied the essential oil of *C. sativum* and they observed that the essential oil of coriander inhibited microorganisms (Baratta *et al.*, 1998; Elgayyar *et al.*, 2001; Larran *et al.*, 2001). Ates and Erdogru, (2003) reported that *Coriandrum sativum* seed extracts showed no inhibition zone to the microorganisms tested like Sagdic *et al.* (2003). However ethyl acetate and acetone extracts used by Ates and Erdogru, (2003), the reason of the different result was plant collection site and bacterial strains different from us. Ates and Erdogru (2003) collected spices from Malatya, but ours from Cine and they tested *B. brevis*, *B. cereus*, *B. megaterium*, *B. subtilis*, *B. subtilis var niger*, *Enterococcus faecalis*, *K. pneumoniae*, *L. monocytogenes*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *P. aeruginosa*, *S. aureus*, *Y. enterocolitica* bacteria.

It is interesting that there are differences in the antibacterial effects of plant groups, due to the phytochemical differences between species and collection site, and also there are differences in the antibacterial effects of microorganisms to some plants, due to the cell wall structure, species and subspecies. According to our results, while clove showed the most effective antibacterial activity, coriander showed the least effective antibacterial activity among the tested plants.

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