



**Full Length Article**

# Antimicrobial Activity of Selected Plant Spices Marketed in the West Anatolia

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## ABSTRACT

Herbs and spices are sources of many bioactive compounds that can improve the taste of foods as well as influence digestion and metabolism processes. The present study was performed to evaluate the antimicrobial activity of ten Turkish medicinal plant spices, used in the traditional system of medicine, against 10 pathogenic bacterial species and yeast, *C. albicans*, using the agar well diffusion method. Anti-candidal activity was detected in 8 plants. Extracts of *Alchemilla vulgaris*, *Laurus nobilis*, *Melissa officinalis*, *Silybum marianum*, *Camellia sinensis* (5a), *Camellia sinensis* (5b), *Rosmarinus officinalis*, *Hibiscus* sp. and *Foeniculum vulgare* showed broad-spectrum antimicrobial activity with inhibition zones ranging from 4 to 32 mm, except *Erica vulgaris*. The most resistant microorganisms were *Escherichia coli* and *Salmonella typhimurium*. The most susceptible organisms were *Kocuria rhizophila* and *Candida albicans*. Minimum Inhibitory Concentrations (MIC) of crude extracts were determined for the three highly active plants showing activity against *Staphylococcus aureus*, *Escherichia coli*; *Kocuria rhizophila*, *Bacillus cereus*, *Enterococcus faecalis* and *Candida albicans*. MICs of active extracts ranged from 2.92 to 10 ≤ mg/mL against one or other test bacteria. © 2010 Friends Science Publishers

**Key Word:** Antimicrobial activity; Spices; Resistant; Microorganisms; West Anatolia

## INTRODUCTION

Additives are harmful for human health, especially monosodium glutamate, aspartame, saccharin, sodium cyclamate, sulfites, nitrates, nitrites and antibiotics. It causes headache, nausea, weakness, mental retarding, seizures, cancer and anorexia (Rangan & Barceloux, 2009). As a result, consumer's interest in natural products, especially plant extracts. Essential oils and/or their components are becoming increasingly popular as natural antimicrobial agents to be used for a wide variety of purposes, including food preservation, complementary medicine and natural therapeutics (Cosentino *et al.*, 2003). Spices and herbs belong to condiments, substances that do not contain nutritive components. Although a few dozens different spice plants are of global importance, many more are used as condiments locally, in the regions of their natural occurrence. Some of these are traded in small quantities and used in ethnic restaurants (Krejpcio *et al.*, 2007).

Spices are used for food additives and folkloric medicine in Turkey. Turkish folkloric medicine comprises numerous herbal prescriptions for therapeutic purposes, which may vary as healing, wounds, treating inflammation due to infection, skin lesions, leprosy, diarrhea, scabies, venereal diseases, snakebite etc. What is interesting however is that, quite often having with same family plants may be used for different diseases? For example

*Rosmarinus officinalis* used for as a laxative, however *Melissa officinalis* used for treatment of headaches.

For many years, control of bacterial infections by inhibiting microbial growth has been a primary approach of antimicrobial chemotherapy. So commercial antimicrobial drugs have been commonly employed as treatment for infectious diseases for many years. However, in recent years the indiscriminate use of these antibiotics has developed multiple resistances and side effects; therefore, more natural antimicrobial substances from plants are desired (Fu *et al.*, 2007). A large number of herbs possess antimicrobial activity (Voravuthikunchai *et al.*, 2004; Mothana & Lindequist, 2005) and some active components of them have become a potential source of new anti-infective agents (Agunu *et al.*, 2005; Buwa & Van Staden, 2006).

Plant extracts have been studied against bacteria, fungi and yeast in the last three decades. During this period, several studies have been conducted on the antimicrobial properties of Turkish medicinal herbs, spices and their derivatives such as essential oils, extracts and decoctions (Dıgırak *et al.*, 2001; Ates & Erdogrul, 2003; Dulger & Gonuz, 2004; Oskay & Sari, 2007; Ugur *et al.*, 2010). Yet, the information, particularly of medicinal plant spices sold in West Anatolia active against some bacteria and *C. albicans*, until recently has not been studied. Therefore, we have selected 10 Turkish medicinal plant spices frequently used in the Turkish folkloric medicine to be screened

against some bacteria and *C. albicans*. The antimicrobial activity was measured using agar well diffusion and microbroth dilution methods.

## MATERIALS AND METHODS

**Sample collection and storage:** Ten spice preparations were obtained from various retail outlets in West Anatolia, including supermarkets, shops and market stalls in Cine, Turkey in 2009. All samples were stored at ambient temperature until initial sample preparation, after which they were stored at 4°C until required for analysis. The average size of sample ranged between 50 g and 250 g. When samples of one kind were received as several packets or jars, all were mixed in a large container to form a homogeneous mixture and a representative sample was then taken. Voucher specimens were deposited in the Herbarium of Botany, Department of Biology, Celal Bayar University. The parts used were the leaves, flowers and in some cases, seeds (Table I).

**Microorganisms and growth conditions:** Test microorganisms included the following bacteria: *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 39628, *Kocuria rhizophila* ATCC 9341, *Bacillus cereus* CCM 99, *Bacillus subtilis* ATCC 6683, *Salmonella typhimurium* CCM 5445, *Proteus vulgaris* ATCC 6997, *Enterococcus faecalis* ATCC 29212, *Enterobacter cloacae* ATCC 13067, *Enterobacter aerogenes* ATCC 13048 and a yeast *Candida albicans* ATCC 10231. Cultures of these bacteria were grown in Mueller-Hinton Broth (Oxoid) at 37°C for 24 h and the studied yeast was incubated in glucose yeast extract broth at 30°C for 48 h. All the microorganisms were obtained from the Department of Biology, Ege University (İzmir/Turkey).

**Preparation of the crude ethanol extracts:** The spices parts were separated, washed with distilled water, dried and powdered finely using a blender. A 25 g of ground air-dried material were shaken in 150 mL of 96% (w/v) ethanol (EtOH 96°) at room temperature with stirring for 4 days (200 cycles/minute). The ethanol was evaporated to dryness after extraction progress. The extract was weighed and dissolved in ethanol (2 mL) at a concentration of 200 mg/mL and stored at 4°C for further experiments.

**Antimicrobial assay:** *In vitro* antimicrobial studies were carried out by the agar well diffusion method (Perez *et al.*, 1990) with slight modification according to the present experimental conditions. Briefly, bacterial strains grown on nutrient agar (37°C for 24 h) and *C. albicans* grown on Potato Dextrose Agar (30°C for 48 h), were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 McFarland standards ( $10^6$  Colony Forming Units/mL). Then, 50 µL inoculums were added to 25 mL melted Mueller Hinton Agar (MHA) for bacteria and Potato Dextrose Agar (PDA) (Oxoid, Basingstoke, UK) for *C. albicans* medium cooled at 45°C. These were then poured into 90 mm diameter Petri dishes and maintained for 1 h at

room temperature. A 6 mm diameter wells were cut in the agar plate and 60 µL of extract concentration (4 mg/well) and pure extraction solvent (EtOH 96°, 60 µL) as a negative control were loaded individually in the wells. The dishes were preincubated at 4°C for 2 h to uniform diffusion into the agar. After preincubation, the plates were incubated at 37°C for 24 h for bacteria and 30°C for 48 h for yeast. The antimicrobial activity was determined by measuring the inhibition zone diameter around the wells. In addition, commercial antibiotics such as nalidixic acid (30 µg), chloramphenicol (30 µg), ampicillin (10 µg), novobiocin (30 µg) and nystatin (10 µg) were used as positive control to determine the sensitivity of the strains. These studies were performed in three times and the results were expressed as average values.

**Determination of minimal inhibitory concentration:** The minimum inhibitory concentration (MIC) was determined for the three highly active spices that showed antimicrobial activity against test microorganisms. The microtiter broth dilution technique with slight modification was performed by using the CLSI standards (CLSI, 2003; CLSI, 2006). A sterile 96 round-bottom well plate was labeled. A volume of 100 µL of extract solution was pipetted into the first row of the plate. To all other wells 50 µL of double strength Mueller Hinton broth or Potato Dextrose broth was added. Serial dilutions were performed using a micropipette (A1-A10). Tips were discarded after use such that each well had 50 µL of the test material in serially descending concentrations. Then, 50 µL of broth containing bacterial suspension ( $5 \times 10^6$  cfu/mL) or yeast ( $5 \times 10^5$  cfu/mL) was added to each well. Each column of wells contained a single antimicrobial extract in progressive dilutions and was inoculated with a single microorganism. Each plate had a set of both a growth (A11) and sterility control (A12). Plates were sealed with clean film to ensure that microorganisms did not become dehydrated. The plates were prepared and placed in an incubator set at 37°C for 18–24 h and at 30°C for 48 h, respectively for bacteria and *C. albicans*. After incubation, added 10 µL of 0.2% 2,3-5 Triphenyl tetrazolium chloride (TTC) solution to each well of microtitre plate. The plates containing TTC were incubated one h at 37°C for reaction. The color change was then assessed visually. Any color changes from purple to pink, which showed the growth of organism. MIC concentration does not exhibit reduction of TTC into formazan so the MIC was defined as that the lowest inhibitory concentration of the antimicrobial agent contained in the microtiter well in which the absence of visual color change (colorless) first observed. The average of six values was calculated and that was the MIC for the test extract and microbial strain.

**Statistical analysis:** The mean values were statistically analyzed using MINITAB Release 13.20 program by the general one-way (unstacked) analysis of variance (ANOVA) to find out the most effective spices extract and the most sensitive test microorganisms.

## RESULTS AND DISCUSSION

The antimicrobial activity of selected plant spices against the mentioned microorganisms and their effectiveness were assessed by the presence or absence of inhibition zones and zone diameter and MIC values. The results are given in Table II and Table III. All plants, except the *E. vulgaris*, studied in this work showed antimicrobial activity against at least one of the test microorganisms, with inhibition zones ranging from 4 to 32 mm (Table II). However, the plants differ significantly in their activity against test microorganisms. The most active plants were *L. nobilis*, *M. officinalis* and *R. officinalis*, which showed broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria and *C. albicans*. On the other hand, the least active plants were *Hibiscus* sp., *S. marianum*, *C. sinensis* (5a), *C. sinensis* (6a) *F. vulgare* and *A. vulgaris*.

The antimicrobial activity of the extracts and their potency was assessed by the presence or absence of inhibition zone as given in Table II. The most susceptible organisms were *K. rhizophila* and *C. albicans*, which were sensitive to eight extracts. *S. aureus*, *B. subtilis* and *E. faecalis* were being sensitive to seven plant extracts, *B. cereus* being sensitive to 6 plant extracts, *P. vulgaris*, *E. cloaceae*, *E. aerogenes* being sensitive to 5 plant extracts. The most resistant species were *E. coli* and *S. typhimurium* being resistant to 6 plant extracts.

Maximum inhibitions were observed with the extract of *M. officinalis* against *K. rhizophila* and *S. aureus* with inhibition zone 32 and 28 mm, respectively. The inhibition zone against *E. coli* produced by the extract of 4 plants i.e., *L. nobilis*, *M. officinalis*, *C. sinensis* and *R. officinalis*. Eight plants, namely, *L. nobilis*, *M. officinalis* and *R. officinalis*, *Hibiscus* sp, *S. marianum*, *C. sinensis* (5a), *C. sinensis* (6a), *A. vulgaris* showed anticandidal activity; the first one was highly active (18 mm).

Similar reports of antibacterial activities of *R. officinalis* leaves showed various inhibitory effects against Gram positive and Gram negative bacteria (7-16 inhibition zone), except the acetone extracts against *Yersinia enterocolitica* (Erdogrul, 2002). Different result of these were explained by Yesil-Celiktas *et al.* (2007), who showed the antimicrobial activities of the essential oils against the tested bacteria differed, depending on location and seasonal variations. In addition, Ozcan and Chalchat (2008) reported that the oil yield of dried plant (volume/dry weight) obtained by hydro-distillation was 1.9%. Twenty compounds representing 99.93% of the oils were identified. The main constituents of the oils were p-cymene, linalool, gamma-terpinene, thymol, beta-pinene, alpha-pinene and eucalyptol. The oil consisted of monoterpenic hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons. It causes antimicrobial activity. In addition, the inhibition effect of rosemary oil was investigated against *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum* (Ozcan & Chalchat, 2008). Similar study by Ertürk (2006) in

relation with antibacterial activities of *L. nobilis* leaves showed various inhibitory effects against Gram positive and Gram negative bacteria, *Aspergillus niger* and *Candida albicans*. In the same study, among the tested microorganisms *C. albicans* showed the highest inhibition zone (25 mm). In our study, *M. officinalis* showed largest inhibition zone (32 mm) against *K. rhizophila*. Amount of applied dose may be affect antimicrobial activity. Ozcan and Erkmén (2001) used three concentrations (1, 10 & 15%) of laurel to test on various microorganisms (*S. typhimurium*, *B. cereus*, *S. aureus*, *Enterococcus faecalis*, *E. coli*, *Y. enterocolitica*, *Saccharomyces cerevisiae*, *C. rugosa*, *Rhizopus oryzae* & *A. niger*). While laurel essential oil showed antimicrobial effects on *B. cereus* and *E. faecalis* at 1 and 10% concentrations, other microorganisms did not.

According to Santoyo *et al.* (2006) antimicrobial activity of *L. nobilis* essential oil content were found active against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* with inhibition zones 25, 20.1, 21.1 and 23 mm, respectively. Our results confirmed this situation (14-24 mm). In addition, Dulger and Gonuz (2004) reported a 10% aqueous dimethylsulfoxide (DMSO) extract of *R. officinalis* inhibited the growth of *S. aureus* and *B. cereus* (15-22 mm), but not *E. coli*, *P. vulgaris* and *C. albicans*. Our results obtained with *R. officinalis* for *E. coli*, *S. aureus* and *C. albicans* were 18, 24 and 14 mm, respectively. According to Mangena and Muyima (1999) essential oil of *R. officinalis* 1:1 and 1:2 concentrations with an inhibition zones 6-14 and 6-10 mm respectively, which confirmed to our results (10-26 mm). Mimica-Dukic *et al.* (2004) reported that 20 and 50% of *M. officinalis* essential oils showed 0-39.8 mm inhibition zone against tested bacteria. Gram-positive bacteria seemed to be more sensitive to the different examined oils than Gram-negatives, as noted here. In a similar study, Pasqua *et al.* (2005) reported that the oil of *M. officinalis* showed low antimicrobial activity against *Lactobacillus*, *Enterococcus*, *Pseudomonas* and *Staphylococcus aureus* strains. They used methanol for the extraction, it may be affect antimicrobial activity. Dissimilar results may be attributed to differences in techniques and extracts, because different methods were used and the variable sensitivity of different microorganisms to chemical substances relates to different resistance levels between the strains (Oskay & Sari, 2007).

Sensitivity of test strains was, in decreasing order: *K. rhizophila* > *S. aureus* > *E. faecalis* > *C. albicans* > *E. aerogenes* > *B. subtilis* > *B. cereus* > *P. vulgaris* = *E. cloaceae* > *E. coli* > *S. typhimurium*. Gram-negative bacteria were less sensitive than Gram-positive, which may be due to their differences in the cell wall composition (Ahmad & Beg, 2001). It was interesting to note that tested bacteria showed more sensitivity to the investigated plant extracts. This clearly indicates that active compounds of these extracts interfere with composition of Gram-negative bacteria cell wall.

Significant antimicrobial effects, expressed as MIC of

**Table I: List of spices screened for antimicrobial activity**

Scientific name/code	Family	Local name	Used part (s)*
<i>Alchemilla vulgaris</i> L./BH-1	Rosaceae	Aslan Pençesi (Kadın Mantosu)	LF
<i>Laurus nobilis</i> L. /BH-2	Lauraceae	Defne	LF
<i>Melissa officinalis</i> L. /BH-3	Lamiaceae	Oğul Otu	LF
<i>Silybum marianum</i> (L.) Gaertn/BH-4	Asteraceae	Kocabaş (Eşek Dikeni)	LF+ FL
<i>Camellia sinensis</i> (L.) Kuntze/BH-5a	Theaceae	Siyah Çay	LF
<i>C. sinensis</i> (L.) Kuntze/BH-6a	Theaceae	Yeşil Çay	LF
<i>Rosmarinus officinalis</i> L./BH-7	Lamiaceae	Biberiye (Kuşdili)	LF
<i>Hibiscus</i> sp. /BH-8	Malvaceae	Japon Güllü (Gül Hatmi)	LF
<i>Foeniculum vulgare</i> Mill./BH-9	Apiaceae	Rezene	SD
<i>Erica vulgaris</i> L./BH-10	Ericaceae	Funda (Süpürgeotu)	LF

\*Used parts: LF-leaves; FL-flowers; SD -Seeds

**Table II: Antimicrobial activity of the ethanol extracts of the spices and some standard antibiotics**

Plant Species	SA <sup>a</sup>	EC	KR	BC	BS	STYP	PV	EF	ECLC	EA	CA
<i>A. vulgaris</i>	12 <sup>b</sup>	0	14	0	0	0	10	12	0	0	10
<i>L. nobilis</i>	18	16	24	14	18	18	16	22	20	14	18
<i>M. officinalis</i>	28	12	32	18	22	14	26	20	14	22	16
<i>S. marianum</i>	0	0	8	0	0	0	0	6	0	0	10
<i>C. sinensis</i> (5a)	10	0	12	8	6	0	0	0	0	0	8
<i>C. sinensis</i> (6a)	12	4	12	10	6	0	0	8	6	8	10
<i>R. officinalis</i>	24	18	22	16	12	10	12	18	16	26	14
<i>Hibiscus</i> sp.	0	0	0	0	6	0	0	0	0	0	0
<i>F. vulgare</i>	16	0	14	10	10	4	8	12	16	12	10
<i>E. vulgaris</i>	0	0	0	0	0	0	0	0	0	0	0
NA <sup>c</sup>	20	26	10 <sup>R</sup>	28	30	6 <sup>R</sup>	12 <sup>R</sup>	28	10 <sup>R</sup>	26	ND
CLH	20	26	30	26	28	40	16	28	28	12 <sup>R</sup>	ND
AMP	15 <sup>R</sup>	0	26	0	10 <sup>R</sup>	0	10 <sup>R</sup>	16	16	6 <sup>R</sup>	ND
NV	32	0	28	25	13 <sup>R</sup>	40	26	28	22	17 <sup>R</sup>	ND
NYS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22
NC <sup>d</sup>	8	2	6	6	4	6	6	4	4	4	2

<sup>a</sup>Microorganisms: SA - *Staphylococcus aureus*; EC - *Escherichia coli*; KR - *Kocuria rhizophila*; BC-*Bacillus cereus*; BS-*Bacillus subtilis*; STYP-*Salmonella typhimurium*; PV-*Proteus vulgaris*; EF-*Enterococcus faecalis*; ECLC-*Enterobacter cloacae*; EA-*Enterobacter aerogenes*; CA-*Candida albicans*

<sup>b</sup>Applied extract dose, 4 mg/well; inhibition zone diameter in mm, not including well diameter (6 mm) and if equal to negative control inhibitions or under recorded as 0; mean values, n = 3; 0 - no inhibitory activity; ND - not determined

<sup>c</sup>Standard antibiotics, NA-Nalidixic acid (30 µg/disc); CHL-Chloramphenicol (30 µg/disc); AMP-Ampicillin (10 µg/disc); NV-Novobiocin (30 µg/disc); NYS - Nystatin (10 µg/disc). Diameter of inhibition zone in mm, including disc diameter (6 mm). <sup>R</sup>-resistant. 6-no activity

<sup>d</sup>NC -negative control, 60 µL 96% ethanol

**Table III: MIC values of selected spices ethanol extracts**

Plant Species	SA <sup>a</sup>	EC	KR	BC	EF	CA
<i>L. nobilis</i>	4.17±1.291 <sup>c</sup>	10≤	6.67±2.58	7.50±2.74	5.83±2.041	10≤
<i>M. officinalis</i>	3.33±1.291	10≤	2.92±1.021	6.67±2.58	4.58±1.021	10≤
<i>R. officinalis</i>	4.56±1.021	9.17±2.041	3.75±1.369	8.33±2.58	7.50±2.74	10≤
NEO <sup>b</sup>	27.08±9.88	5.73±2.067	3.37±1.235	7.29±2.471	6.25±2.344	ND
GE	3.91±1.172	6.25±2.344	2.87±1.034	5.73±2.067	4.43±0.781	ND
NYS	ND	ND	ND	ND	ND	0.49±0.293

<sup>a</sup>SA - *Staphylococcus aureus*; EC-*Escherichia coli*; KR-*Kocuria rhizophila*; BC-*Bacillus cereus*; EF-*Enterococcus faecalis*; CA-*Candida albicans*

<sup>b</sup>Standard antibiotics: NEO-Neomycin; GE-gentamycin; NYS-Nystatin. ND-not determined. MIC for ethanol extract and standard antibiotics expressed as mg/ml and µg/ml respectively

<sup>c</sup>Data presented as the mean value of six determinations ± standard deviation.

crude extracts, were observed against *S. aureus*, *E. coli*; *K. rhizophila*, *B. cereus*; *E. faecalis*, *C. albicans* (Table III). The maximal MIC values for bacterial strains, sensitive to plant extracts, were in the range of 2.92-10≤ mg/mL. Among the plants tested, ethanolic extract of *M. officinalis* showed very strong activity against *S. aureus* with the best MIC of 3.33 mg/mL. The lowest MIC obtained with *M. officinalis* extract was 2.92 mg/mL for *K. rhizophila*, whereas the highest one was 10≤ mg/mL for *M. officinalis*

and *L. nobilis* extracts against *E. coli*. MIC value of *R. officinalis* for *E. coli* was 9.17 mg/mL.

Recently, there is a growing interest in finding natural antioxidants and/or antimicrobial compounds as an alternative to synthetic substances, which are commonly used in the food industry. Many studies have reported that plants contain a wide variety of compounds with beneficial health effects. It has generally been the essential oils of these plants rather than their extracts that have had the

greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin (Rios & Recio, 2005). Particularly, many herbs and spices used to aromatize foods have been screened as sources of natural antioxidants and antimicrobial compounds (Santoyo *et al.*, 2006). The side effect of antibiotics, increasing in antibiotics resistant microorganisms and the high cost of production of chemical compounds, drug companies are now looking for new alternatives. Some researchers explained that medicinal plants could be used for pharmaceutical area (Kokoska *et al.*, 2002; Wannissorn *et al.*, 2005). With the increasing awareness of people towards natural food and natural therapies, spices might act as the most obvious alternative (Sofia *et al.*, 2007).

## CONCLUSION

The antibacterial and anti-candidal activities vary with the plant species. Antimicrobial activity may be dependent on the cell wall composition of bacteria. The use of investigated plant extracts could be beneficial for human health and budget. However, more information is imperative about antimicrobial properties of the plants to different bacteria.

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