APTEFF, 40, 1-220 (2009) UDC: 664.5:582.28+577.18
DOI: 10.2298/APT0940195S BIBLID: 1450-7188 (2009) 40, 195-209
Review

ANTIMICROBIAL EFFECTS OF SPICES AND HERBS ESSENTIAL OILS

Marija M. Škrinjar and Nevena T. Nemet

Spices and herbs have been used as food additives since ancient times, as flavouring agents but also as natural food preservatives. A number of spices shows antimicrobial activity against different types of microorganisms. This article gives a literature review of recent investigations considering antimicrobial activity of essential oils widely used spices and herbs, such as garlic, mustard, cinnamon, cumin, clove, bay, thyme, basil, oregano, pepper, ginger, sage, rosemary etc., against most common bacteria and fungi that contaminate food (Listeria spp., Staphylococcus spp., Salmonella spp., Escherichia spp., Pseudomonas spp., Aspergillus spp., Cladosporium spp. and many others). Antimicrobial activity depends on the type of spice or herb, type of food and microorganism, as well as on the chemical composition and content of extracts and essential oils. Summarizing results of different investigations, relative antimicrobial effectiveness can be made, and it shows that cinnamon, cloves and mustrad have very strong antimicrobial potential, cumin, oregano, sage, thyme and rosemary show medium inhibitory effect, and spices such as pepper and ginger have weak inhibitory effect.

KEY WORDS: Spices, herbs, antimicrobial, antifungal activity

INTRODUCTION

In the recent years, consumers have become more concerned about the processed food they eat. Syntehtic preservatives, which have been used in foods for decades, may lead to negative health consequences (1). Besides, the use of synthetic compounds have significant drawbacks, such as increasing cost, handling hazards, concerns about residues on food and threat to human environment (2). Therefore, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. Those are, in the first place, extracts and essential oils (EOs) of spices and herbs (3). As natural foodstuffs, spices and herbs appeal to all who question safety of synthetic food additives and demand high-quality products that at the same time are safe and stable (4).

Spices and herbs have been added to food since ancient times, not only as flavouring agents, but also as folk medicine and food preservatives (5-7). Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of mil-

Dr. Marija M. Škrinjar, Prof., skrinjarm@uns.ac.rs, Nevena T. Nemet, B.Sc., nevenan@uns.ac.rs, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia

lions of people all over the world. They are essentially flavouring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties (8). Nowadays, plenty of spices and herbs are valued for their antimicrobial activities and medicinal effects in addition to their flavour and fragrance qualities (9).

Spices and herbs

There is no particular definition of spices, mostly because they are derived from different parts of the plants, such as cardamom from seed, bay leaf from leafs, clove from flower bud, pepper from fruit, cinnamon from bark or ginger from rhyzome. Furthermore, there is no a common method to classify spices. They can be clasiffied by their flavour and colour, i.e., hot (pepper), pungent (garlic), aromatic (cinnamon, clove), colouring (turmeric) and herbaceous (rosemary, sage), or according to their taste, such as sweet, spicy, sour, bitter and astringent (10).

Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including foodborne pathogens (5, 11, 12). It has been reported that spices owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (13). As reviewed by López-Malo et al. (14), some of antimicrobial components that have been identified in spices and herbs are: eugenol from cloves, thymol from thyme and oregano, carvacrol from oregano, vanillin from vanilla, allicin from garlic, cinnamic aldehyde from cinnamon, allyl isothiocyanate from mustard, etc.

Among these products, particular interest has been focused on essential oils (EOs) and their components (15), because they are known to be active against a wide variety of microorganisms, including food-borne pathogens and spoilage bacteria (16, 17). EOs are aromatic and volatile oily liquids of an aromatic plant's secondary metabolism. They are normally formed in special cells or groups of cells, found in leaves and stems, and commonly concentrated in one particular region such as leaves, bark or fruit (18). EOs have long served as flavouring agents in food and beverages, but they are much more important because of their antimicrobial activity, which is assigned to a number of small molecules of terpenoids and phenolic compounds (thymol, carvacrol, eugenol) (19, 20).

Antibacterial activity of EOs

Nedorostova et al. (21) identified antibacterial properties of EOs in vapour phase against five foodborne bacteria - *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Staphylococcus aureus.* In vitro antibacterial activity of 27 EOs in vapour phase was evaluated by modified disc volatilization method (22) at eight different concentrations (0.0042–0.53 µl/cm^3), and the minimum inhibitory concentrations (MICs) were recorded. The MIC was expressed as microlitres of EOs per volume unit of atmosphere above the organism growing on the agar surface, and it was defined as the lowest concentration which made clearly visible inhibition zone. Results are summarised at Table 1.

Thirteen of the 27 EOs were active at least against one bacterial strain in the range of tested concentrations. The most effecient was *Armoracia rusticana* (horseradish), which inhibited both Gram-positive and Gram-negative strains with MIC 0.0083 μl/cm^3, and *Allium sativum* (garlic), which was significantly more active against Gram-positive (MICs 0.0083 μl/cm^3) than against Gram-negative (MICs 0.26–0.53 μl/cm^3) bacteria. It is important to notice that *P.aeruginosa* was inhibited only by these two, horseradish and garlic, EOs. *S. aureus* was the most susceptible bacterium (inhibited by all active EOs), followed by *E. coli* > *L.monocytogenes* > *S. enteritidis* > *P. aeruginosa*, which was the least inhibited. In general, Gram-negative strains were somewhat less inhibited than Gram-positive strains. Findings of this research suggest that horseradish, garlic, oregano, marjoram, savory, thyme, large thyme and wild thyme EOs are highly effective in vapour phase and could be potentially used to fight against foodborne bacterial pathogens.

Table 1. MICs (μl/cm^3) of essential oils in vapour phase effective against foodborne bacteria (21)

Plant species	**Yield %**	**Gram-positive**		**Gram-negative**		
		LM	**SA**	**EC**	**PA**	**SE**
Allium sativum	0.33	0.0083	0.0083	0.53	0.53	0.26
Armoracia rusticana	0.03	0.0083	0.0083	0.0083	0.0083	0.0083
Caryopteris x clandonensis	0.15	-	0.53	-	-	-
Hyssopus officinalis	0.16	-	0.53	-	-	-
Mentha x villosa	0.83	-	0.53	-	-	-
Nepeta x faassenii	0.33	-	0.53	-	-	-
Ocimum basilicum var. Grant verte	0.08	-	0.53	-	-	-
Origanum majorana	0.53	-	0.53	0.26	-	-
Origanum vulgare	0.8	0.066	0.017	0.066	-	0.13
Satureja montana	0.28	0.26	0.033	0.033	-	0.26
Thymus pulegoides	0.15	0.26	0.033	0.033	-	0.26
Thymusseryllum	0.27	0.53	0.033	0.033	-	-
Thymus vulgaris	0.23	0.26	0.017	0.033	-	0.033

Yield – in % (v/w) of fresh weight; LM – *Listeria monocytogenes*; SA – *Staphylococcus aureus*; EC – *Escherichia coli*; PA – *Pseudomonas aeruginosa*; SE – *Salmonella enteritidis*.

Zhang et al. (23) studied the antibacterial properties of 14 EOs (clove, oregano, rosemary, pepper, nutmeg, liquorice, turmeric, aniseed, cassia bark, fennel, prickly ash, round cardamom, dahurian angelica root and angelica) against four common meat spoilage and pathogenic bacteria (*Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens* and *Lactobacillus sake*) and their results showed that individual extracts of clove, rosemary, cassia bark and liquorice contained strong antibacterial activity, but the mixture of rosemary and liquorice extracts was the best inhibitor against all four types of microbes. These herb extracts are widely used in the food industry and are generally regarded as safe (GRAS). Hence, they may be considered as natural preservatives acceptable by the food industry.

There has been several more general studies about antimicrobial activity of EOs. One of them is the work of Gutierrez et al. (24), who evaluated EOs of lemon balm, marjoram, oregano and thyme, and applied them on food model media based on lettuce, milk and meat. Minimum inhibitory concentrations were determined against *Enterobacter* spp., *Listeria* spp., *Lactobacillus* spp. and *Pseudomonas* spp. According to the results, shown in Table 2, the average efficacy of EOs against *Listeria* spp. was in the following order: oregano>thyme> lemon balm, while the efficacy order of EOs against the spoilage bacteria was: oregano>thyme> marjoram.

Table 2. MIC of EOs used in this study against the selected bacteria in TSB (A), lettuce leaf model media (B) or beef extract (C) (24)

Microorganism	**Oregano**	**Thyme**	**Marjoram**	**Lemon balm**
(A)				
Enterobacter cloacae	400	600	6000	ND[a]
Listeria innocua NCTC11288	200	200	ND	2500
Listeria monocytogenes IL323	200	200	ND	2500
Listeria monocytogenes NCTC1194	200	200	ND	2500
Pseudomonas fluorescens	2000	2000	50 000	ND
Pseudomonas putida	2000	2000	50 000	ND
(B)				
Enterobacter cloacae	250	250	2000	ND
Listeria innocua NCTC11288	20	30	ND	250
Listeria monocytogenes IL323	20	30	2000	ND
Pseudomonas fluorescens	250	250	2000	ND
(C)				
Listeria innocua NCTC11288	60	125	ND	500
Listeria monocytogenes NCTC1194	60	125	ND	500
Pseudomonas fluorescens	1500	2500	12500	ND
Pseudomonas putida	1500	2500	12500	ND

[a] ND, not determined.

As has been shown by some other researchers, the use of antimicrobials can reduce or eliminate specific microorganisms but it may also produce favourable conditions for other microorganisms (25). It is recognized that this situation is less likely to develop towards substances that have more than one mode of action (26), so it is suggested that the antimicrobial activity of EOs is attributed to more than one mechanism (27). Thus, combining EOs could lead to useful efficacy against both spoilage and pathogenic target organisms. Gutierrez et al. (24) tested the synergy of EO combinations and expresed it as fractional inhibitory concentration (FIC) index, in the lettuce leaf model media. The FIC indices were calculated as FIC_A+FIC_B, where $FIC_A=MIC_{A,combination}/MIC_{A,alone}$ and $FIC_B=MIC_{B,combination}/MIC_{B,alone}$. The results were interpreted as synergy (FIC< 0.5), addition (0.5<FIC< 1), indifference (1 <FIC<4) or antagonism (FIC> 4). See Table 3.

Table 3. FIC values of EOs combinations in lettuce leaf model media (24)

EO combination	*Enterobacter cloacae*	*Pseudomonas fluorescens*	*Listeria innocua* NCTC11288	*Listeria monocytogenes* IL323
Oregano+marjoram	1.75 (I)	2.00 (I)	ND[a]	ND
Oregano+lemon balm	ND	ND	1.50 (I)	1.25 (I)
Oregano+thyme	0.75 (A)	0.88 (A)	1.00 (A)	1.18 (I)
Thyme+marjoram	1.00(A)	1.38 (I)	ND	ND
Thyme+lemon balm	ND	ND	0.75 (A)	1.25 (I)

Results are interpreted as synergy (S, FIC< 0.5), addition (A, 0.5 <FIC<1), indifference (I, 1<FIC< 4) or antagonism (AN, FIC> 4).
[a] ND, not detected.

Results from Table 3 show that - with reference to the FIC scale - no synergistic effect (<0.5) was found, but addition occurred with a number of combinations. More incidence of additive effects was found with EO combinations against *Listeria* strains. Combinations of oregano with thyme or lemon balm were more effective against *Listeria monocytogenes*. The combination of thyme with lemon balm had greater efficacy against *Listeria innocua*. Only one combination (oregano with thyme) had additive effects against both spoilage microorganisms. No antagonism was observed for any of the combinations evaluated. These results can be observed as an addition to some earlier studies of the same author, Gutierrez et al. (28), considering the synergistic effect of marjoram and thyme with some other EOs (Table 4).

Table 4. FIC indices of marjoram and thyme EOs combinations against *L. monocytogenes* IL323 (28)

Combinations	**Marjoram**	**Thyme**
Marjoram or thyme +		
Basil	0.75 (A)	0.94 (A)
Lemon balm	1.25 (I)	1.25 (I)
Marjoram	-	1.55 (I)
Oregano	1.18 (I)	1.18 (I)
Rosemary	1.03 (A)	1.06 (A)
Sage	1.00 (A)	1.00 (A)
Thyme	1.55 (I)	-

.

For successful applications of EOs in different food systems, potential interactions between EOs and food components have to be determined. There is a number of examples where some studies have shown that plant extracts are useful for reduction of pathogens in some food product, while others reported very low antimicrobial activity or no effect when the same EOs were applied to other product. Thus, the application of plant EOs requires the evaluation of efficacy within food products or in model systems that closely mimic food composition, because the efficacy of many antimicrobials may be reduced by certain food components (29). This was also investigated by Gutierrez et al.

(28). In this complex task, they studied the effect of food ingredients and pH on the antimicrobial efficacy of EOs using a number of model media and *L.monocytogenes* IL323 as indicator strain. The EOs used in the study were oregano (30 ppm) and thyme (60 ppm), and they were assessed independently. Results, expressed as lag phase duration and maximum specific growth rate of *L. monocytogenes*, are shown in Table 5.

Table 5. Lag phase duration (λ) and maximum specific growth rate (μmax) of *L. monocytogenes* IL323 grown in model media containing oregano (30 ppm) or thyme (60 ppm) (28)

Model media	Oregano		Thyme		Control[a]	
	Λ [h]	μmax[h^{-1}]	Λ [h]	μmax[h^{-1}]	Λ [h]	μmax[h^{-1}]
Beef extract						
1.5	7.39	0.034	9.15	0.079	8.01	0.054
3.0	7.81	0.076	11.79	0.146	6.14	0.074
6.0	10.75	0.099	10.94	0.195	6.48	0.117
12.0	10.79	0.195	9.75	0.200	6.33	0.215
Starch media						
0.0	15.01	0.185	12.06	0.250	7.80	0.238
1.0	13.96	0.208	11.69	0.271	7.04	0.268
5.0	9.71	0.142	8.87	0.164	7.58	0.147
10.0	8.84	0.098	8.43	0.104	8.04	0.097
Sunflower oil media						
0.0	15.23	0.240	15.05	0.231	7.75	0.279
1.0	14.21	0.226	12.54	0.209	7.31	0.223
5.0	10.50	0.208	9.22	0.235	7.24	0.200
10.0	9.17	0.174	9.47	0.220	7.33	0.170
pH						
TSB pH4	0.00	0.000	0.00	0.000	0.00	0.000
TSB pH5	12.43	0.004	10.13	0.016	9.55	0.017
TSB pH6	7.70	0.141	9.48	0.175	6.75	0.173
TSB pH7	9.50	0.284	10.80	0.328	6.88	0.261

[a] *Listeria monocytogenes* grown in model media without any EO was used as the control. Model media comprised the following: (a) water soluble starch from potato in TSB; (b) beef extract in deionized water; and (c) sunflower oil in TSB. The pH of each model medium was adjusted to 7.2. To determine the effect of pH on EO efficacy TSB was adjusted to pH 4, 5, 6 or 7 with 1 M HCl solution

The lag phase of *Listeria monocytogenes* grown in beef extract containing oregano was longer than the control at protein concentrations of 6% and 12%. The efficacy of oregano and thyme was greater at higher concentrations of protein, probably because peptones from beef extract may have hydrophobic properties which facilitate dissolution of EOs in this medium.

In addition, the growth rate of *L. monocytogenes* decreased at higher starch concentrations. The EO efficacy was reduced at high concentrations of starch, in contrast to the general observation that carbohydrates in foods do not protect bacteria from the action of EOs as much as fat and protein do (30).

The growth of *L. monocytogenes* was monitored in model media containing four different sunflower oil concentrations (0, 1, 5 and 10%), in order to determine effect of oil on the antimicrobial activity. High concentrations of sunflower oil had a negative influence on the antimicrobial activity of oregano and thyme EOs.

Effect of pH was evaluated using TSB at pH 4, 5, 6 and 7. *L. monocytogenes* did not grow at pH 4. The lag phase of *Listeria* grown in pH model media with EO was longer than in EO free controls, especially at pH 5, however, the lag phase was also greatest at pH 5 in control media. The growth rate of *L. monocytogenes* increased at higher pH values, regardless of the presence or absence of EOs. Some previous studies showed that the inhibitory effect of plant extracts was greater at acidic pH values (31). The susceptibility of bacteria to EOs appears to increase at lower pH since the hydrophobicity of EOs increases at low pH, enabling consequently easier dissolution in the lipids of the cell membrane of target bacteria (32).

Antifungal activity of EOs

All mentioned investigations have demonstrated anibacterial properties of EOs against pathogenic and spoilage bacteria in food. However, the presence and growth of fungi in food may also cause spoilage and result in a reduction in quality and quantity (33-39). As reported by a number of authors (38-45), some *Aspergillus* species are responsible for many cases of food and feed contamination, and *Aspergillus flavus* and *Aspergillus parasiticus* are able to produce aflatoxins in food and feedstuffs, which are known to be potent hepatocarcinogens in animals and humans (46). *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare are major storage fungi found regularly in important cereal grains cultivated and stored throughout the world (47), which produce aflatoxins B_1, B_2, G_1 and G_2. The biosynthesis of aflatoxins can be inhibited by extracts and EOs from certain plants toxic to fungi and can control the fungal growth and mycotoxin production.(48).

Omidbeygi et al. (49) evaluated the antifungal activity of three EOs (thyme, summer savory and clove) in culture medium and as a real system in tomato paste (in vitro and in vivo). Results clearly showed that in vitro each EO had notable antifungal activity. Thyme EO has the highest antifungal activity, followed by summer savory and clove EOs. Complete inhibition of growth of *Aspergillus flavus* was observed at 350 and 500 ppm of thyme and summer savory EOs, respectively, while 500ppm of clove oil had inhibition of 87.5%. In vivo studies was performed in tomato paste, and while in vitro experiments showed that 500 and 350ppm of thyme oil could inhibit the growth of *A. flavus* completely (100%), inhibition in tomato paste were 87% and 42%, respectively. Also, in other treatments inhibition in tomato paste was lower than in culture medium. It has probably been related to the more complex matrix of tomato paste than culture medium. However, the need to use plant EOs at higher concentrations in food than in laboratory media is believed to be due to the more complex growth environment in food, which provides microbial cells with greater protection from antimicrobial agents.

Atanda et al. (50), evaluated essential oils of sweet basil (*Ocimum basilicum*), cassia (*Cinnamomum cassia*), coriander (*Coriandrum sativum*) and bay leaf (*Laurus nobilis*) for their potential in the growth control of aflatoxigenic fungus *Aspergillus parasiticus* CFR

APTEFF, 40, 1-220 (2009) UDC: 664.5:582.28+577.18
DOI: 10.2298/APT0940195S BIBLID: 1450-7188 (2009) 40, 195-209
Review

223 and aflatoxin production. Results, expressed as mycelia dry weight and aflatoxin production in basal medium, are summarised in Table 6.

Table 6. Effect of different concentrations of spice essential oil on the mycelia dry weight and aflatoxin production in basal medium (52)

Essential oil	**Concentration [%v/v]**	**Mycelia dry weight [g/ml]**	**Aflatoxin [µg/ml]**	
			B_1	**G_1**
Cassia	0	0.50	0.050	0.046
	1	0.38	0.029	0.027
	2	0.40	0.012	0.011
	3	0.48	0.004	0.003
	4	0.52	0.002	0.003
	5	0.55	0.001	0.001
Sweet basil	1	0.20	0.045	0.042
	2	0.20	0.040	0.038
	3	0.20	0.038	0.035
	4	0.20	0.024	0.020
	5	0.00	0.000	0.000
Coriander	1	0.50	0.050	0.046
	2	0.50	0.050	0.046
	3	0.50	0.050	0.046
	4	0.50	0.050	0.046
	5	0.50	0.050	0.046
Bay leaf	1	0.55	0.050	0.046
	2	0.55	0.045	0.042
	3	0.65	0.040	0.038
	4	0.70	0.030	0.035
	5	0.75	0.023	0.020

The EOs of sweet basil inhibited completely mycelia growth and prevented aflatoxin formation at the concentration of 5% (v/v). At the same concentration, oils of cassia and bay leaf reduced the aflatoxin concentration (B_1+G_1) of the fungus to 0.002 µg/ml (97.92%) and 0.043 µg/ml (55.21%), respectively. Cassia and bay leaf EOs stimulated the mycelia growth of the fungus, while coriander oil did not have any effect on either the mycelia growth or aflatoxin content of the fungus.

Tzortzakis et al. (51), tested antifungal activity of lemongrass (*Cympopogon citratus L.*) oil (range concentration between 25 and 500 ppm) against *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer* and *Aspergillus niger* in vitro. Oil-enrichment resulted in significant reduction of the subsequent colony development for the examined microorganisms. Fungal spore production was inhibited up to 70% at 25 ppm of lemongrass oil concentration when compared with control samples (equivalent plates stored in the ambient air). At the highest oil concentration (500 ppm), fungal sporulation was completely retarded (Table 7). Lemongrass oil reduced spore germination and germ tube length of *C. coccodes*, *B. cinerea*, *C. herbarum* and *R. stolonifer* with the effects dependent on oil concentration. This study indicated that EOs may possess antifungal activity and can be exploited as an ideal treatment for future plant disease management programs eliminating fungal spread.

Viuda-Martos et al. (52), investigated the effect of the essential oils of lemon (*Citrus lemon L.*), mandarin (*Citrus reticulata L.*), grapefruit (*Citrus paradisi L.*) and orange (*Citrus sinensis L.*) on the growth of moulds commonly associated with food spoilage: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Penicillium verrucosum*, using the agar dilution method. The essential oils of lemon, orange, mandarin and grapefruit at the concentrations assayed (0.27-0.94%) all showed the capacity to reduce or inhibit the growth of the named moulds. The growth of *A. niger* was completely inhibited when a concentration of 0.94% of any of the EOs was used. Orange EO produced the greatest reduction in mycelium growth with this fungus, and it is followed by lemon EO, then the mandarin, while grapefruit EO caused the lowest percentage of mycelial reduction in *A. niger.* In the case of *A. flavus*, efficacy of EOs was in the following order: mandarin>lemon>grapefruit>orange, while total inhibition of growth was obtained with all the EOs at the highest concentrations of 0,94%. The same concentration for growth inhibition was found for *P.verrucosum* and *P.chrysogenum,* and the order of oil efficacy against these moulds was grapefruit>lemon>orange>mandarin.

Table 7. Impacts of lemongrass (*Cympopogon citratus* L.) essential oil-enrichment on spore production (number of spores×10^6) of tested fungi grown on PDA (51)

EO concentration [ppm]	***Colleotrichum coccodes***	***Botrytis cinerea***	***Cladosporium herbarum***	***Rhizopus stolonifer***	***Aspergillus niger***
0	12.10	14.28	79.35	14.13	169.50
25	5.13	4.30	47.70	9.23	99.35
50	2.55	5.23	45.38	8.63	62.93
100	1.53	5.75	39.88	5.00	63.13
500	0.00	0.00	0.00	0.00	0.00

In the study of Vilela et al. (53), the *Eucalyptus globulus* Labill. EO and its major compound 1,8-cineole were evaluated for antifungal activity against *A. flavus* and *A. parasiticus*, as well as on aflatoxin production. Results of this study showed that *Eucalyptus globulus* oil had clear dose-dependent antifungal activity on both fungal species. Complete fungal growth inhibition was verified at the concentration of 50 ml oil per millilitre of medium in the contact assay. The antifungal activity offered by 1.8 cineole only showed effects at the highest concentration tested, which indicated the major oil constituent is not the only component responsible for limiting fungal growth. Inhibition of aflatoxin B_1 production required a higher oil dose than was required for inhibition of fungal growth.

Škrinjar et al. (54) examined an inhibitory effect of various concentrations (0.0, 0.5, 1.0, 1.5 and 2.0%) of mint (*Mentha piperita* L.) and caraway (*Carvum carvi* L.) on the growth of some toxigenic *Aspergillus* species and aflatoxin B_1 production. Mint showed

DOI: 10.2298/APT0940195S BIBLID: 1450-7188 (2009) 40, 195-209

stronger inhibitory effect than caraway. Concentrations of 1.0. 1.5 and 2.0% reduced the growth of all tested *Aspergillus* species, which was poor and hardly visible, while concentration of 2% of caraway was needed to achieve the same effect. The applied concentrations of mint and caraway inhibited completely the production of AB_1 by *Aspergillus flavus*.

Yeasts are widely distributed in nature and are able to spoil many foods such as wines, cheese, vinegar, beverages, juices, fruits, salads, sugar and meat, causing changes in odour, colour, taste and texture (55). Many of data indicate the EOs inhibitory effects of various spices and herbs on these microorganisms.

The study of Souza et al. (56), aimed at verifying the effectiveness of oreganum (*Origanum vulgare* L.) EO to inhibit the growth/survival of various food spoiling yeasts. Results, expressed in millimeters of yeast growth inhibition halos, are shown in Table 8.

Table 8. *Origanum vulgare* L. essential oil MIC on food spoiling yeasts determined by solid medium diffusion[a] (56)

Yeasts	***Origanum vulgare* L. essential oil [µl/ml]**							
	160	80	40	20	10	5	2.5	1.25
Candida albicans	38	28	20	12	10	0	0	0
Candida krusei	32	25	15	12	0	0	0	0
Candida tropicalis	35	27	21	14	11	0	0	0
Pichia minuscula	39	36	31	21	16	11	0	0
Pichia ohmeri	33	28	16	13	10	0	0	0
Rodotorula rubra	38	34	30	28	14	0	0	0
Saccharomyces cerevisae	26	22	14	11	0	0	0	0

[a] Results expressed in millimeters of yeast growth inhibition halos.

The results showed that the EO had a substantial inhibitory effeect on all assayed yeast strains, noted by large growth inhibition halos. Most assayed strains showed an MIC of 10 µL/mL. The highest inhibitory activity was observed against *P. minuscula* (the lowest MIC of 5 µL/mL) and the largest growth inhibition halos. On the other hand, *S. cerevisae* and *C. krusei* were the least sensitive yeasts with an MIC of 20 µL/mL; however *S. cerevisae* showed the smallest growth inhibition halo diameters when compared to all other strains. This high antimicrobial activity of *O. vulgare* EO supports the results found by other researchers (57,58).

CONCLUSIONS

Food contamination is enormous public health problem, but it could be controlled by the use of natural preservatives such as essential oils obtained from spices. The fact that many EOs possess antimicrobial activity has been proved by plenty of investigations in the recent years. The type and optimal concentration of EO depend on the product used and against which species of bacteria or fungi it is to be used. But if EOs are expected to be widely applied as antibacterials and antifungals, the organoleptical impact should be considered as the use of naturally derived preservatives can alter the taste of food or exceed acceptable flavour thresholds. The problem may occur if high concentrations required to achieve useful EO antimicrobial activity, result in unacceptable levels of flavours and odours. Therefore, research in this area should be focused on the optimization of EO combinations and applications to obtain effective antimicrobial activity at sufficiently low concentrations so as not to adversely influence the organoleptic acceptability of the foods.

REFERENCES

1. Namiki, M.: Antioxidant/antimutagens in food. Food Sci. Nutr. **29** (1990) 273–300.
2. Paster, N. and L. B. Bullerman: Mould spoilage and mycotoxin formation in grains as controlled by physical means. Int. J. Food Microbiol. **7** (1988) 257−265.
3. Smid, E.J. and L.G.M. Gorris: Natural antimicrobials for food preservation, in Handbook of Food Preservation. Ed. M.S. Rahman, Marcel Dekker, New York (1999) pp. 285–308.
4. Brul, S. and P. Coote: Preservative agents in foods: Mode of action and microbial resistance mechanisms. Int. J. Food Microbiol. **50** (1999), 1-17.
5. Beuchat, L.R.: Antimicrobial properties of spices and their essential oils, in Natural Antimicrobial Systems and Food Preservation. Eds. Y. M. Dillon and R. G. Board, CAB International, Oxon, (1994) pp. 167–179.
6. Nakatani, N.: Antioxidative and antimicrobial constituents of herbs and spices, in Spices, Herbs and Edible Fungi. Ed. G. Charalambous, Elsevier Science, New York, (1994) pp. 251–271.
7. Cutler, H.G.: Natural product flavor compounds as potential antimicrobials, insecticides, and medicinals. Agro-Food Ind. Hi-Tech **6**, (1995) 19–23.
8. Oluwafemi, F.: Correlation between dietary aflatoxins and human male infertility, Ph.D. Thesis, University of Benin, 2000.
9. Shan, B., Y. Cai, J. D. Brooks and H. Corke: The in vitro antibacterial activity of dietary spice and medicinal herb extract. Int. J. Food Microbiol. **117** (2007) 112-119.
10. Ceylan, E. and D.Y.C. Fung: Antimicrobial activity of spices. J. Rapid Meth. Aut. Mic. **12** (2004) 1–55.
11. Smith-Palmer, A., J. Stewart and L. Fyfe: Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett. Appl. Microbiol. **26** (1998) 118–122.
12. Hara-Kudo, Y., A. Kobayashi, Y. Sugita-Konishi and K. Kondo: Antibacterial activity of plants used in cooking for aroma and taste. J. Food Protect. **67** (2004) 2820–2824.
13. Ebana, R. U. B., B.E. Madunagu, E.D. Ekpe and I. N. Otung: Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocymoides*, *Kola nitida* and *Citrus aurantiofolia*. J. App. Bacteriol. **71** (1991) 398–401.
14. López-Malo, A., J. Barreto-Valdivieso, E. Palou and F. S. Martin: *Aspergillus flavus* growth response to cinnamon extract and sodium benzoate mixtures. Food Control **18** (2006) 1358−1362.
15. Elgayyar, M., F.A. Draughon, D.A. Golden and J.R. Mount: Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Protect. **64**, 7 (2001) 1019–1024.

16. Hammer, K. A., C.F. Carson and T. V. Riley: Antimicrobial activity of essential oils and other plant extracts. J. App. Microbiol. **86** (1999) 985–990.
17. Dorman, H. J. D. and S. G. Deans: Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. App. Microbiol. **88** (2000) 308–316.
18. Oussalah, M., S. Caillet, L. Saucier and M. Lacroix: Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control **18**, 5 (2006) 414–420.
19. Conner, D. E.: Naturally occurring compounds, in Antimicrobials in foods. Eds. P. Davidson and A. L. Branen, Marcel Dekker, Inc. New York (1993) pp. 441–468.
20. Didry, N., L. Dubreuil and M. Pinkas: Activité antimicrobienne du thymol, du carvacrol et de l'aldéhyde cinnamique seuls ou associés (Antibacterial activity of thymol, carvacrol, and cinnalmaldehyde singly or in combinations). Pharmazie **48** (1993) 301–308.
21. Nedorostova, L., P. Kloucek, L. Kokoska, M. Stolcova and J. Pulkrabek: Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. Food Control **20** (2009) 157-160.
22. Lopez, P., C. Sanchez, R. Batlle and C. Nerin: Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. J. Agr. Food Chem. **53** (2005) 6939–6946.
23. Zhang, H., B. Kong, Y.L. Xiong and X. Sun: Antimicrobial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4°C. Meat Sci. **81** (2009) 686-692.
24. Gutierrez, J., C. Barry-Ryan and P. Bourke: Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. Food Microbiol. **26** (2009) 142-150.
25. Davidson, P.M. and A.L. Branen: Food antimicrobials – an introduction, in Antimicrobial in Food. Eds. P.M. Davidson, J.N. Sofos, and A.L. Branen, A.L., CRC Press, Taylor and Francis Group ed. (2005) pp. 1–9.
26. Ippolito, A. and F. Nigro: Natural antimicrobials in postharvest storage of fresh fruits and vegetables, in Natural Antimicrobials for the Minimal Processing of Foods. Ed. S. Roller, Woodhead Publishing LTD (2003) pp. 201–223.
27. Moreira, M.R., A.G. Ponce, C.E. Del Valle and S.I. Roura: Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT–Food Sci. Tech. **38**, 5 (2005) 565–570.
28. Gutierrez, J., C. Barry-Ryan and P. Bourke: The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol. **124** (2008) 91-97.
29. Glass, K.A. and E.A. Johnson: Antagonistic effect of fat on the antibotulinal activity of food preservatives and fatty acids. Food Microbiol. **21** (2004) 675–682.
30. Shelef, L.A., E.K. Jyothi and M.A. Bulgarelli: Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. J. Food Sci. **49** (1984), 737-740.
31. Del Campo, J., M.J. Amito and C. Nguyen-The: Antimicrobial effect of rosemary extracts. J. Food Protect. **63**, 10 (2000) 1359–1368.

32. Juven, B.J., J. Kanner, F. Schved and H. Weisslowicz: Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. App. Bacteriol. **76** (1994) 626–631.
33. Škrinjar M., I. Kolarović, G. Dimić and O. Jovanović: Pojava plesni i nekih mikotoksina u sirovinama korišćenim u konditorskoj industriji. Žito hleb **27**, 1 (2000) 9-14.
34. Škrinjar, M., I. Vujković, O. Jovanović, I. Kolarević and G. Dimić: Mogućnost gljivičnog kvara industrijski punjenog kolača sa većim sadržajem vlage. Žito hleb **27**, 3 (2000) 93-97.
35. Škrinjar, M., V.Došen-Bogićević and I. Vujković: Mycopopulations, aflatoxins and ochratoxin in some semi-dry sausage, 47th International Congress of Meat Science and Technology, Krakow, 21-31 August 2001, Proceedings 7-P27, pp. 272-273.
36. Škrinjar, M: Are toxigenic moulds and mycotoxins frequent contaminants of wheat flour?, 3rd International Congress «Flour-Bread», Opatija, 26-29 October 2005, Book of Abstracts, p. 10.
37. Škrinjar, M: Uljarice – mogući nosioci mikotoksina. Uljarstvo **36**, 1-2 (2005) 47-50.
38. Škrinjar, M. and D. Tešanović: Hrana u ugostiteljstvu i njeno čuvanje, Prirodno-matematički fakultet, Univerzitet u Novom Sadu, Novi Sad (2007).
39. Šarić, Lj. and M. Škrinjar: Share of aflatoxigenic moulds from genera *Asspergillus* and *Penicillium* in mycopopulations isolated from spices for meat processing industry. Proceedings for Natural Sciences **114** (2008) 115-122.
40. Abarc, M. L., M. R. Bragulat, G. Castella, and F. J. Cabanes: Myco-flora and aflatoxin producing strains in animal mixed feeds. J. Food Protect. **57** (1994) 256–258.
41. Škrinjar, M., M. Bandu, E. Dimić, K. Bjelobaba and R. Romanić: Infekcija semena suncokreta žetve 2006. aflatoksigenim gljivama. Uljarstvo **38**, 1-2 (2007) 41-48.
42. Šarić, Lj. and M. Škrinjar: Proizvodnja aflatoksina B1 od strane plesni izolovanih iz začina koji se koriste u industriji mesa, International 54th Meat Industry Conference, Vrnjačka Banja, 18-20 June 2007, Book of Abstracts, p. 129.
43. Škrinjar, M: Fungal and mycotoxin contamination of some food industry byproducts, 25th Food Technology Days, Ljubljana, 17-18 April 2008, pp. 73-84.
44. Škrinjar, M., M. Ač, M. Krajinović and A. Popović-Vranješ: Zdravstveni i ekonomski značaj prisustva toksigenih plesni i mikotoksina u stočnoj hrani. Savremena poljoprivreda **57**, 3-4 (2008) 26-34.
45. Guo, B. Z., J.S. Russin, R.L. Brown, T.E. Celveland and N. W. Widstrom: Resistance to aflatoxin contamination in corn as influenced by relative humidity and kernel germination. J. Food Protect. **59** (1996) 276–281.
46. Dvorackova, I. : Aflatoxins and human health. Boca Raton, FL: CRC Press (1990).
47. Frisvad, J.C.: Mycotoxins and Mycotoxigenic Fungi, in Storage Grain Ecosystems. Eds. D.S. Jayas, N.D.G. White, W.E. Muir, Marcel Dekker, New York, (1995) pp. 251–258.
48. Pinto, M.M., E. Gonçales, M.H. Rossi, J.D. Felı´cio, C.S. Medina, M.J.B. Fernandes and L.C. Simoni: Activity of the aqueous extract from *Polymnia sonchifolia* leaves on growth and production of aflatoxin B_1 by *Aspergillus flavus*. Braz. J. Microbiol. **32** (2001) 127–129.

49. Omidbeygi, M., M. Barzegar, Z. Hamidi and H. Naghdibadi: Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control **18** (2007) 1518–1523.
50. Atanda, O.O., I. Akpan and F. Oluwafemi: The potential of some spice essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. Food Control **18** (2007) 601–607.
51. Tzortzakis, N. G. and C. D. Economakis: Antifungal activity of lemongrass (*Cympopogon citratus L.*) essential oil against key postharvest pathogens. Inn. Food Sci. Emerg. Technol. **8** (2007) 253–258.
52. Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernández-López and J. Pérez-Álvarez: Antifungal activity of lemon (*Citrus lemon L.*), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Control **19** (2008) 1130–1138.
53. Vilela, G. R., G.S. Almeida, M. Aparecida Bismara Regitano D'Arce, M.H. Duarte Moraes, J.O. Brito, M.F. das G.F. da Silva, S.C. Silva, S.M. de Stefano Piedade, M.A. Calori-Domingues and E. Micotti da Gloria: Activity of essential oil and its major compound, 1,8 cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. J. Stored Prod. Res. **45** (2009) 108-111.
54. Škrinjar, M., A. Mandić, A. Mišan, M. Sakač, Lj. Šarić and M. Zec: Effect of mint (*Mentha piperita* L.) and caraway (*Carvum carvi* L.) on the growth of some toxigenic *Aspergillus* species and aflatoxin B1 production. Proc. Nat. Sci, Matica Srpska Novi Sad **116** (2009) 131-139.
55. Ray, B.: Fundamental food microbiology. Boca Raton: CRC Press (1996).
56. Souza, E.L., T.L.M. Stamford, E.O. Lima and V.N. Trajano: Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. Food Control **18** (2007) 409–413.
57. Skandamis, P., E. Tsigarida and G. J. Nychas: The effect of oregano essential oil on survival/death of *Salmonella typhimurium* in meat stored at 5 °C under aerobic, VP/MAP conditions. Food Microbiol. **19** (2002) 97–103.
58. Chun, S. S., A.V. Vattern, Y.T. Lin and K.. Shetty: Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. Process Biochem. **40** (2004) 809–816.

АНТИМИКРОБНО ДЕЛОВАЊЕ ЕСЕНЦИЈАЛНИХ УЉА ЗАЧИНА И ЛЕКОВИТОГ БИЉА

Марија М. Шкрињар и Невена Т. Немет

Зачини и лековито биље користе се као додаци храни још од давнина, у својству ароме, као побољшивачи укуса а такође и као природни конзерванси. Велики број зачина и лековитог биља показује антимикробно и антифунгално деловање према одређеним микроорганизмима. Овај рад даје литературни преглед нових истраживања која се тичу наведене активности есенцијалних уља широко распрострањених зачина и лековитог биља, као што су бели лук, слачица, цимет, кумин, каранфилић,

ловор, мајчина душица, босиљак, оригано, бибер, ђумбир, жалфија, рузмарин и др., против најчешћих бактерија и гљива које контаминирају храну (*Listeria* spp., *Staphylococcus* spp., *Salmonella* spp., *Escherichia* spp., *Pseudomonas* spp., *Aspergillus* spp., *Cladosporium* spp. и многи други). Антимикробна активност зависи од врсте зачина, врсте намирница и микроорганизама на које се примењује, као и од хемијског састава и концентрације екстраката или есенцијалних уља зачина. Сумирањем резултата добијених од стране различитих аутора, може се извести закључак о релативној антимикробној и антифунгалној ефикасности одређених зачина и лековитог биља, према коме цимет, каранфилић и слачица имају веома јак антимикробни потенцијал, кумин, оригано, жалфија, мајчина душица и рузмарин имају средњи, док бибер и ђумбир имају слаб инхибиторни ефекат.

Received 23 July 2009
Accepted 15 October 2009