African Journal of Biotechnology Vol. 11(94), pp. 16065-16070, 22 November, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3058
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Comparative antimicrobial activity of clove and fennel essential oils against food borne pathogenic fungi and food spoilage bacteria

Shabnam Javed[1]*, Sobia Mushtaq[1], Ibatsam Khokhar[1], Rauf Ahmad[2] and M. Saleem Haider[1]

[1]Institute of Agriculture, University of the Punjab, Quaid-e- Azam Campus, Lahore-54590, Pakistan.
[2]FBRC, PCSIR Laboratories Complex, Ferozepur Road Lahore, Pakistan.

Accepted 6 June, 2012

Antifungal and antibacterial activities of essential oils obtained from fennel seeds (*Feoniculum vulgare* Mill) and clove buds (*Syzygium aromaticum*) were studied by agar well dilution technique. Both essential oils (EOs) from fennel and clove exhibited pronounced and varying degrees of growth inhibition against fungal (86 to 39%) and bacterial pathogens (42 to 20%). Fennel oil depicted significant and greater fungitoxicity in case of three fungal strains *Alternaria alternate* (7.7, 3.8 cm) *Fusarium oxysporum* (5.9, 4.1 cm) and *Aspergillus flavus* (4.5, 3.7 cm) except two *Aspergillus* strains, *Aspergillus acculeatus and Apergillus fumigatus* where clove oil showed greater inhibition zone (5.5, 5.9 cm) (3.5, 3.7 cm) respectively. *A. alternate* was found to be most sensitive strain, which growth was suppressed up to 86% by fennel seeds oil. Bactericidal activity of culinary spices was evaluated against five food spoilage bacteria namely: *Pseudomonas syringae, Bacillus subtilis, Escherichia coli, Staphylococcus* sp*.,* and *Aeromicrobium erythreum.* Fennel oil was found fairly active against bacterial strains as compared to clove oil with highest antibacterial activity against Gram positive bacteria *Bacillus subtilis* (3.8 cm) and least against Gram negative bacteria *E. coli* (2.2 cm). The summarizing results from the present investigation showed that fennel seeds oil is a relatively stronger antimicrobial agent against broad range of pathogens as compared to clove oil, except in case of certain *Aspergillus* strains and *E. coli.*

Key words: Food spoilage, anti-bacterial, pathogenic fungi, clove, fennel, essential oil.

INTRODUCTION

Food-borne diseases are still a major problem in the world, in spite of the modern improvements in food hygiene, even in well-developed countries (WHO, 2002). A variety of microorganisms lead to food spoilage and food borne diseases. In the advance stage preservation, preservatives must be used to prevent the growth of spoiling microbes in the food industry (Sagdıc and Ozcan, 2003). In foods for decades, synthetic preservatives have been used that may lead to negative health consequences, such as convert ingested materials into toxic substances, increasing cost, handling hazards, residues on food that threat to human environment (Namiki, 1990; Paster and Bullerman, 1988; Farag et al., 1989). Consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives have led to exploring naturally-occurring antimicrobials for food preservation (Gould, 1995). Essential oils (EOs) of spices and herbs at first place are safe and stable as natural foodstuffs have been added to food since ancient times. Spices essential oils appeal to all who question safety of synthetic food additives and at the same time demand high-quality (Nakatani 1994; Cutler et al., 1995). Kitchen spices used in daily life also used in traditional medicines to treat infectious diseases seem to be a potent source of new bioactive secondary metabolites. The antimicrobial effects of spices are mostly due to the essential oils present in their composition (Arora-Dlijit and Kaur, 1999).

*Corresponding author. shabnamjaved@yahoomail.com.

Essential oils, derived from kitchen spices (for example, fennel (*Foeniculum vulgare*), ajowin (*Trachyspermum ammi)*, peppermint (*Menthapiperita*), Kalonji (*Nigella sativa*), thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*) have been reported to possess bactericidal, fungicidal and viricidal activity (Beuchat, 1994; Nakatani, 1994; Cutler, 1995; Morsi, 2000). Fennel (*Foeniculum vulgare* Mill) and Cloves (*Syzygium aromaticum*) have been used for centuries as spice to enhance the flavor, aroma of foods and their medicinal values (Beaux et al., 1997; Tanira et al., 1996). Fennel and cloves are traditional part of Pakistani, Chinese, Arabic and Western medicine. Essential oils possess phenolic components known to possess antimicrobial activity and some are generally recognized as safe (GRAS) substances and therefore could be used in food to prevent post-harvest growth of native and contaminant fungi and bacteria (Singh et al., 2002; Moreira et al., 2005).

Fennel (*Foeniculum vulgare* Miller), with a sweet, earthy flavor which belongs to the family Apiaceae has long been used as herbal remedy. Medicinally, fennel is used as analgesic, antioxidant, antispasmodic, anti-inflammatory, carminative, and diuretic (Oktay et al., 2003; Mimica-Dukic et al., 2003). Recently, antimicrobial activities of fennel seed extracts and essential oils has been investigated and explored value of this commonly used kitchen spice (Ozcan et al., 2006; Mata et al., 2007) .Essential oil of Fennel (EOF) can be used as possible bio fungicides, alternative to synthetic fungicides against phytopathogenic fungi (Soylu et al., 2007).The essential oil and extracts of clove (EOC) are used as a topical application to relieve pain and to promote healing, anti-aging, cardiovascular disease, arthritis , infections (skin, flu, bacterial, viral and fungal, hepatitis, parasitic), digestive problems (nausea, vomiting, diarrhea), skin cancer, thyroid dysfunction and also finds use in the fragrance and flavouring industries (Chaieb et al., 2007; Liu et al., 1997; Kim et al., 1998; Zheng et al., 1992; Cai and Wu, 1996). Strong antimicrobial activity of in clove essential oil is referred to the rich amount of eugenol. This phenolic compound is capable to denature proteins and reacts phospholipids of cell membrane alternating their permeability (Briozzo, 1989; Deans and Ritchie, 1987). Gulfraz et al. (2008) showed inhibition of fennel oil against *Bacillus cereus, Bacillus magaterium, Bacillus pumilus, Bacillus substilis, Eschericha coli, Klebsiella pneumonia, Micrococcus lutus, Pseudomonos pupida, P. syringae, and Candida albicans* as compared to its ethanolic and methanolic extracts. Eugénia et al. (2009) evaluated the minimum fungicidal concentration of the clove oil and its main component, eugenol, against *Candida, Aspergillus* and dermatophyte clinical and American Type Culture Collection strains. It has been proved that clove oil and eugenol have considerable antifungal activity against clinically relevant fungi, including fluconazole-resistant strains.

Regarding the safety of food and human health, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. The purpose of the present study was to evaluate the antimicrobial effectiveness of the essential oils of Fennel and clove functioning both as flavoring and additive.

MATERIALS AND METHODS

Aromatic spices

Fennel seeds and Clove buds were purchased from Local market in Lahore city (Pakistan) during March, 2010. The spices were identified by The Flora of Pakistan (Nasir and Ali, 1978). The voucher specimen PU.IAGS.HHC.635 and PU.IAGS. HHC.636 was given to sweet fennel seeds (*Foeniculum vulgare* Mill.) and clove [*Syzygium aromaticum* (L.) Merrill and Perry], respectively and deposited in the Herbarium of Institute of Agricultural Sciences, IAGS, University of the Punjab (PU) Lahore, Pakistan. The seeds and buds were ground using pestle and mortar and packed in polythene bags and placed in a dried place for further oil extractions.

Essential oil (EO) extraction

For oil extraction, 1 kg ground of each spices material was subjected to hydrodistillation for 4 h to obtain essential oil. The residue was removed by filtration through filter paper. The essential oils were dried over anhydrous sodium sulfate and stored in black vials at 5°C. Essential oils yield was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of EO recovered}}{\text{Weight of spices}} \times 100$$

Each essential oil dilution (60 mg/ml) was prepared in dimethylsulphoxide (DMSO), followed by sterilization using a 0.45 µm membrane filter.

Microbial strains

The EOs of clove and fennel was tested against a range of 10 microorganisms, collected from First Fungal Culture Bank of Pakistan, (FCBP), Institute of Mycology and Plant Pathology, University of The Punjab Lahore Pakistan (Table 1). These fungal and bacterial cultures were maintained on Malt Extract Agar (MEA 2%) and nutrient agar (NA) at 4°C respectively and revived on fresh medium for further study. Fungal inoculum was prepared from 7 days old culture in sterilized distilled water with help of hematocyotmeter (10^8 CFU/ml). Each bacterial inoculum was prepared from 24 days old culture (10^5 CFU/mL; 0.5 Mac-Farland).

Agar-well diffusion assay

Standardized inoculum of fungi and bacteria (100 µL) each was spread onto a malt extract Agar (MEA) and nutrient Agar (NA) plates respectively with the help of sterile spreader. The inoculated plates were allowed to dry and sterile cork borer of diameter 8.0 mm was used to bore wells in center of inoculated agar plates. Subsequently, a 60 µL volume of oil of test spices were introduced in wells. Sterile DMSO served as the control. The plates were allowed to stand for 1 h to diffuse and then incubated at 37°C for 24 h for bacteria and for 5 days for fungi. The zone of inhibition was recorded to the nearest size in cm. Percentage inhibition and index of antifungal activity was calculated with the following formulas

Table 1. List of fungal and bacterial species.

Acculeatus	Fungal specie	Substrate
188	*Alternaria alternata* (Fr.) Keissl.	*Arachis hypogaea* leaf
346	*Fusarium oxysporum* Schltdl.	Soil
596	*Aspergillus fumigatus* Fresen.	*Malus domestica* fruit
862	*Aspergillus flavu,* Link	Strawberry fruit,
1009	*Aspergillus aculeatus* Iizuka	Rhizosphere of guava
217	*Aeromicrobium erythreum* Miller	Canal water
009	*Pseudomonas syringae*	Cherry fruit,
189	*Bacillus subtilis*	Chiku rhizospheric soil
123	*E. coli*	*Pisum sativum* legume
294	*Staphylococcus*. sp.	Textile industrial effluent

$$\% \text{ Inhibition} = \frac{\text{Mycelium of control (cm)} - \text{Experimental}}{\text{Myceliun of control (cm)}}$$

$$\text{Antifungi index} = \frac{1 \quad - \text{ Experimental mycelium growth (cm)}}{\text{Myceliun of control (cm)}}$$

Statistical analysis

The antimicrobial activity was determined by measuring the diameter of zone of inhibition which was statistically analyzed by applying Duncan's Multiple Range Test (Steel and Torrie, 1980) using computer software COSTAT.

RESULTS

The yield obtained of clove and fennel seed oils were 17.46 and 0.72 % respectively. Both spice EOs showed considerable inhibitory potential against fungi (39 to 86%) as well as bacteria (24 to 42%) against all the test food borne pathogens (Tables 2 and 3). The results from the well diffusion method indicated that *A. alternate* (86%) and *B. subtilis* (42%) were the most sensitive microorganisms tested, showing the largest inhibition zones 7.7 and 3.8 cm respectively. However, fennel seeds overall depicted far greater antimicrobial potential than clove oil where (7.7, 3.8 cm) (5.9, 4.1 cm) (4.5, 3.7 cm) (3.8, 2.2 cm) (3.3, 2.4 cm) (2.3, 2.1 cm) (2.5, 2.4 cm) zone of inhibition were calculated against *A. alternata*, *A. flavus*, *B. subtilis*, *P. syringae, Staphylococcus* sp. and *Aeromicrobium erythreum* respectively. In case of fungi fennel oil maximum suppressed the growth of *A. alternate* (86%); *A. fumigatus* was found to be least inhibited (39%). Bactericidal activity was maximum in case of *B. subtilis* (42%) and minimum in *E. coli* (24%). Clove oil significantly inhibited the food borne pathogens comparable with that of fennel oil in case of *A. acculeatus* (65, 61%)*, Aspergillus fumigatus* (42, 39%) and *E. coli* (29, 24%). *A. acculeatus* was found to be most sensitive with 5.9 cm inhibition zone and *E. coli* (2.6 cm) fungal and bacteria strain respectively, in case of clove oil.

DISCUSSION

Food borne pathogenic fungi and food spoilage bacteria cause damage to human health reveal drug resistance due to inadequate use of antibiotics. The main advantage of natural sources is that they do not enhance the antibiotic resistance, commonly encountered with the long-term use of synthetic antibiotics (Nenad et al., 2007). Thus, there is a need for the discovery of novel substances from natural sources, including spices. In the present study, the antimicrobial activity of the two spices fennel and clove was evaluated against a panel of food borne pathogens. Fennel oil and clove oil significantly suppressed the microbial growth of all tested five fungal strains with inhibition zone (3.5 to 7.7 cm) and five bacterial strains (2.1 to 3.8 cm) (Tables 2 and 3)..

As far as the antifungal results are concerned, fennel oil showed higher inhibition against *Alternaria alternate* (86, 42%) *Fusarium oxysporum* (65, 42%) and *Aspergillus flavus* (51, 41%) as compared to clove oil whereas, clove oil displayed greater pathogenicity in case of *A. fumigatus* (42, 39%) *and Aspergillus acculeatus* (65, 61%) than fennel oil. Other workers also reported fennel oil as week antifungal for *Aspergillus* species than clove oil (Sunita and Rai, 2008). Overall fennel oil displayed highest antifungal potential against *A. alternate while clove oil inhibited the A. acculeatus* (Table 2).

Antibacterial potential was observed by both spices oil samples with (2.1 to 3.8 cm) inhibition zone. Fennel oil showed pronounced inhibitory effect against three bacterial strains especially against Gram positive bacteria *B. subtilis* (3.8, 2.2 cm), *Pseudomonas syringae* (3.3, 2.4 cm) *Staphylococcus* sp. (2.3, 2.1 cm) and *A. erythreum* (2.5, 2.4 cm) while clove oil in case of Gram negative bacteria *E. coli* (2.6, 2.2 cm) (Table 3). Our results are in line with previous study of Gulfraz et al. (2008) where

Table 2. Effect of Fennel (*F. vulgare*) and cloves (*S. aromaticum*) essential oils on food born pathogenic fungal growth.

Fungal strain	Zone of inhibition		Experimental mycelium growth		Inhibition ratio (%)		Index	
	Fennel oil	Clove oil	Fennel oil	Clove oil	Fennel oil	Clove oil	Fennel oil	Clove oil
Aspergillus flavus	4.5[d]	3.7[f]	4.4 ± 0.39	5.3 ± 0.55	51	41	0.93 ± 0.47	0.58 ± 0.057
Aspergillus fumigatus	3.5[g]	3.7[f]	5.5 ± 0.45	5.2 ± 0.618	39	42	0.60 ± 0.051	0.57 ± 0.068
Aspergillus acculeatus	5.5[c]	5.9[b]	3.5 ± 0.02	3.1 ± 0.173	61	65	0.39 ± 0.005	0.34 ± 0.017
Fusarium oxysporum	5.9[b]	4.1[e]	3.1 ± 0.173	4.9 ± 0.152	65	45	0.34 ± 0.017	0.54 ± 0.015
Alternaria alternata	7.7[a]	3.8[f]	1.2 ± 0.028	5.2 ± 0.057	86	42	0.13 ± 0.002	0.5 ± 0.020

Table 3. Effect of Fennel (*F. vulgare*) and cloves (*S. aromaticum*) essential oils on food spoilage bacteria.

Bacterial strain	Zone of inhibition		Experimental mycelium growth		Inhibition ratio (%)		Index	
	Fennel oil	Clove oil	Fennel oil	Clove oil	Fennel oil	Clove oil	Fennel oil	Clove oil
Pseudomonas syringae	3.3[b]	2.4[de]	5.7 ± 0.28	6.6 ± 0.081	37	26	0.36 ± 0.017	0.27 ± 0.23
Bacillus subtilis	3.8[a]	2.2[fg]	5.2 ± 0.144	6.8 ± 0.087	42	24	0.41 ± 0.005	0.25 ± 0.01
E. coli	2.2[fg]	2.6[c]	6.8 ± 0.087	6.4 ± 0.1	24	29	0.25 ± 0.11	0.29 ± 0.005
Staphylococcus sp.	2.3[ef]	2.1[g]	6.7 ± 0.115	7.2 ± 0.057	25	20	0.25 ± 0.017	0.20 ± 0.001
Aeromicrobium erythreum	2.5[cd]	2.4[de]	6.6 ± 0.086	6.6 ± 0.150	27	26	0.71 ± 0.086	0.73 ± 0.01

fennel oil showed strong inhibitory potential against *Bacillus cereus*, *B. magaterium*, *B. pumilus*, *B. substilis*, *E. coli*, *K. pneumonia*, *M. lutus*, *Pseudomonos pupida*, *P. syringae*, and *Candida albicans* as compared to methanolic and ethanolic seed extracts. *F. vulgare* seed oil is rich in *trans*-anethole and other compounds which are effective against microbes.

Similarly, Kawther (2007) investigated essential oil of fennel plant roots, stem, leaves and seeds against commonly encountered *Candida* species and reported that only the essential oil from seeds of fennel displayed anticandidal activity against *C. albicans* (20 mm) *C. albicans* (20 mm) and *C. tropicalis* (18 mm) where no inhibition zone was recorded in the case of fennel root, stem and leaves. Anwar et al. (2009) analysed fennel oil by gas chromatography-mass spectrometery (GC–MS) and revealed the presence of 23 compounds, with *trans*-anethol (69.87%) as major component followed by fenchone (10.23%), estragole (5.45%) and limonene (5.10%). Ozcan et al. (2006) reported estragole (61.08%), fenchone (23.46%) and limonene (8.68%), respectively as the major constituents in the essential oil of bitter fennel (*F. vulgare* spp. *piperitum*).

The antifungal activity of the clove oil and its main component, eugenol (85.3%) were investigated by Eugénia et al. (2009) against *Candida, Aspergillus* and dermatophyte clinical and American Type Culture Collection strains. The EO and eugenol showed inhibitory activity against all the tested strains. Mainly, phenolic components of essential oils are considered responsible for the antimicrobial activity, followed by aldehydes, ketones, and alcohols (Azzouz and Bullerman, 1982: Shelef et al., 1983: Akgul, 1989). It is difficult to attribute the activity of natural essential oils which are complex mixtures to a particular constituent, it is reasonable to assume that the activity of clove oil can be related to the presence of a high concentration (85.3%) of eugenol. Different modes of action are involved in the antimicrobial activity of EOs (Burt, 2004). The activity may, in part, be due to their hydrophobicity, responsible for their partition into the lipid bilayer of the cell membrane, leading to permeability alteration and a consequent leakage of cell contents. As typical lipophiles, essential oils can travel through the cell wall and cytoplasmic membrane, disrupt the structure of the different layers of polysaccharides, fatty acids and phospholipids, and permeabilize them (Burt, 2004). Many reports Bakkali et al., 2008; Pasqua et al., 2007; (Hammer et al., 2004) suggest that antimicrobial mechanism is because of membrane damage. Bacteriostatic action involving permeabilization of the membranes is associated with ion loss, loss of proton, depletion of ATP and reduction of membrane potential. Clove oil and eugenol also considerably reduce the quantity of ergosterol which is a specific fungal cell membrane component responsible for maintaining cell function and integrity (Rodriguez et al., 1985). Germ tube formation by microbes albicans was completely or almost completely inhibited by clove oil and eugenol concentrations below the MIC values (Eugénia et al., 2009).

Conclusion

Fennel (*F. vulgare*) and cloves (*S. aromaticum*) were found to have important antimicrobial activity against the food borne pathogenic fungi and food spoilage bacteria. In this regard the use of culinary spices fennel seeds and clove buds as natural preservatives in food products are valuable not only for increasing shelf life of foodstuffs but it could be a future target for replacing chemical additives and synthetic antimicrobial agents.

ACKNOWLEDGEMENTS

The authors are thankful to (HHC) Herbal Heritage Centre and (FCBP) Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan for providing facilities of essential oil extraction and microbial strains.

REFRENCES

Akgul A (1989). Antimicrobial activity of black cumin (*Nigella sativa* L.) essential oil. J Gazi Pharmacol Faculty. 6: 63–68.

Anwar F, Alia M, Hussain AI, Shahid M (2009). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan, Flavour Fragr. J. 24: 170–176.

Arora-Daljit S, Kaur J (1999). Antimicrobial activity of spices. Int. J. Antimicrob. Agents. 12:257-262.

Azzouz MA, Bullerman LB (1982).Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. J Food Protect .45:1298–1301.

Bakkali F, Averbeck S, Averbeck D,Idaomar M(2008).Biological effects of essential oils – a review, Food Chem Toxicol. 46:446–475.

Beaux D, Fleurentin J, Mortier F(1997). Diuretic action of hydroalcohol extract of *Foeniculum vulgare* var. *dulce* (D.C.) roots in rats, Phytotherapy Research. 11: 320–322.

Beuchat LR (1994). 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: 167–179.

Briozzo J (1989). Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. J. Applied Bacteriol. 66: 69-75.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods – a review, Int J Food Microbiol. 94: 223–253.

Cai L, Wu CD (1996). Compounds from *Syzygium aromaticum* processing growth inhibitory activity against oral pathogens. J. Nat. Prod., 59: 987-990.

Chaieb KH, Hajlaoui T, Zmantar KAB, Nakbi M, Rouabhia K, Mahdouani, Bakhrouf A (2007). The chemical composition and biological activity of essential oil, *Eugenia cryophyllata* (*Syzygium aromaticum* L. Myrtaceae), Phytotherapy Research – Wiley InterScience. 21(6): 501-506.

Cutler HG (1995). Natural product flavor compounds as potential antimicrobials, essential oils to reduce a foodborne pathogen, LWT–Food Sci. Tech. 38(5):565–570.

Eugénia P, Vale-Silva L, Cavaleiro C,Salgueiro L (2009). Antifungal activity of the clove essential oil from *Syzygium aromaticum* on*Candida*, *Aspergillus* and dermatophyte species, J Med Microbiol **.** 58(11): 1454-1462**.**

Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA(1989). Antimicrobial activity of some Egyptian spice essential oils, J.Food Protect. 52(9): 665-667.

Gould GW (1995). Homeostatic mechanisms during food preservation by combined methods. In G. Barbosa-Canovas & J. Welti-Chanes (Eds.). Food preservation by moisture control. Lancaster: Techromic Publishing Co. Inc: 397–410.

Gulfraz M, Mehmood S, Minhas N, Jabeen N, Kausar R, Jabeen K and Arshad G(2008). Composition and antimicrobial properties of essential oil of *Foeniculum vulgare,*African Journal of Biotechnology.7 (24): 4364-4368.

Hammer KA, Carson CF, Riley TV (2004). Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans, Candida glabrata* and *Saccharomyces cerevisiae,* J Antimicrob. Chemother. 53: 1081–1085.

Kawther FA (2007).Antimicrobial Activity of Essential Oils of some Medicinal Plants from Saudi Arabia, Saudi J. Biological Sciences. 14 (1): 53-60.

Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH, Shin TY (1998). Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rat. J. Ethnopharmacol., 60: 125-131.

Liu H, Mao P, Hong S (1997). Study on the virus function of extracorporeal restraining HCMV by Chinese traditional medicine *Eugenia caryophyllata* Thunb. Med. J. Chin. People's Liberation Army, 1: 73-75

Mata AT, Proenca C, Ferreira AR, Serralheiro MLM, Nogueira JMF, Araujo MEM (2007). Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices, Food Chem.103: 778.

Mimica-Dukic N, Kujundzic S, Sokovic M, Couladis M(2003). Phytother. Essential oil composition and antifungal activity of *Foeniculum vulgare* Mill. obtained by different distillation conditions. 9: 552.

Moreira MR, Ponce AG, Del Valle CE, Roura SI (2005).Inhibitory parameters of essential oils to reduce a foodborne pathogen, LWT–Food Sci. Tech. 38. 5: 565–570.

Morsi NM (2000). Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibioticresistant bacteria. Acta Microbiol. Polonica, 49(1): 63-74.

Nakatani N (1994). Antioxidative and antimicrobial constituents of herbs and spices, in Spices, Herbs and Edible Fungi. Ed. G. Charalambous, Elsevier Science, New York: 251–271.

Namiki M (1990). Antioxidant/antimutagens in food, Food Sci. Nutr. 29**:** 273–300.

Nenad V, Tanja M, Slobodan S, Slavica S (2007). Antimicrobial activities of essential oil and methanol extract of *Tenvicum montanum*, Comple alternat medic eCAM 4: 17-20.

Oktay M, Gulcin I, Ufrevioglu OI, *Lebensm* (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Wiss. Technol., 36: 263.

Ozcan MM, Chalchat JC, Arslan D, Ates A, Unver A (2006). Comparative essential oil composition and antifungal effect of bitter fennel (*Foeniculum vulgare* ssp. piperitum) fruit oils obtained during different vegetation. J. Med Food. 9(4):552-561.

Pasqua D, Betts R, Hoskins G, Edwards N, Ercolini M, Mauriello G (2007). Membrane toxicity of antimicrobial compounds from essential oils, J Agric Food Chem.55: 4863–4870.

Paster N, Bullerman LB (1988). Mould spoilage and mycotoxin formation in grains as controlled by physical means, Int. J. Food Microbiol., 7: 257−265.

Sagdıc O, Ozcan M (2003). Antibacterial activity of Turkish spice

hydrosols, Food Control. 14.:141–143.
Shelef LA (1983). Antimicrobial effects of spices, J. Food Safety. 6:29–44.
Singh N, Singh RK, Bhunia AK, Stroshine RL (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. Lebensmittel- Wissenschaft und -Technologie, 35: 720–729.
Soylu S, Yigitbas H, Soylu EM, Kurt S (2007). Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. J. Appl. Microbiol. 103: 1021-1030.
Steel RGD, Trorrie HJ (1981).Principles And Procedures of Statistics *A. biometrical* approach. MC Graw Hill international book company.
Sunita B, Rai M (2008). Antifungal Activity of Essential Oils from Indian Medicinal Plants against Human Pathogenic *Aspergillus fumigatus* and *A. niger*, World J. Medical Sciences, 3 (2): 81-88.
Tanira MOM, Shah AH, Mohsin A, Ageel AM, Qureshi S (1996). Pharmacological and Toxicological investigations on *Foeniculum vulgare* Mill., dried fruit extract in experimental animals. Phytotherapy Res. 10: 33-36.
WHO (2002). World Health Report. Reducing Risks, Promoting Healthy Life. 30 October 2002. World Health Organization, Geneva.
Zheng GQ, Kenney PM, Lam LK (1992). Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. J. Nat. Prod., 55: 999-1003.