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

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Antifungal and antibacterial activities of essential oils obtained from fennel seeds (Feniculum 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 alternata (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 aculeatus and Aspergillus fumigatus where clove oil showed greater inhibition zone (5.5, 5.9 cm) (3.5, 3.7 cm) respectively. A. alternata 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 (Sagdic 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).

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Essential oils, derived from kitchen spices (for example, fennel (*Foeniculum vulgare*), ajowan (*Trachyspermum ammi*), peppermint (*Mentha piperita*), Kalonji (*Nigella sativa*), thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*) have been reported to possess bactericidal, fungicidal and viricidal activity (Beuchat, 1994; Nakatan, 1994; Cutler, 1995; Morsi, 2000). Fennel (*Foeniculum vulgare* Mill) and Cloves (*Syzygium aromaticum*) have been used for centuries as spices 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 (Chaleib et al., 2007; Liu et al., 1997; Kim et al., 1998; Zheng et al., 1992; Cai and Wu, 1996). Strong antimicrobial activity of 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 subtilis*, *Eschericha coli*, *Klebsiella pneumonia*, *Micrococcus latus*, *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 preserv-atives 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 dimethyl sulphoxide (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 hemacytometer (10⁶ CFU/ml). Each bacterial inoculum was prepared from 24 days old culture (10⁵ 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.

<table>
<thead>
<tr>
<th>Myceliun of control (cm) – Experimental</th>
<th>Mycelium of control (cm)</th>
<th></th>
<th>% Inhibition</th>
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<tbody>
<tr>
<td>Mycelium of control (cm)</td>
<td>1 - Experimental mycelium growth (cm)</td>
<td>Mycelium of control (cm)</td>
<td></td>
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<tr>
<td>% Inhibition =</td>
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Antifungi index = Mycelium of control (cm) 

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

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 alternata (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 Aspergillus fumigatus (42, 39%) and Aspergillus aculeatus (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. aculeatus (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 Bacillus subtilis (3.8, 2.2 cm), Pseudomonas syringae (3.3, 2.4 cm) Staphylococcus sp. (2.3, 2.1 cm) and Aeromicrobium 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 Gulfrizz et al. (2008) where...
pholipids, and gas leaves. recorded in tropicalis albicans of fennel displayed anticandidal activity against and reported that only the essential oil from seeds against albicans Pseudomonos pupida

Aeromi Staphylococcus E. coli Bacillus subtilis Pseudomonas

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

Pseudomonas syringae 3.3\(^{a}\) 2.4\(^{ab}\) 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\(^{ab}\) 5.2 ± 0.144 6.8 ± 0.087 42 24 0.41 ± 0.005 0.25 ± 0.01

E. coli 2.2\(^{ab}\) 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\(^{ed}\) 2.1\(^{a}\) 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\(^{ab}\) 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. lutas, 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 antieulycal 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 spectrometry (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 antifulgal 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 Bulleman, 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)

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

Aspergillus flavus 4.5\(^{a}\) 3.7\(^{d}\) 4.4 ± 0.39 5.3 ± 0.55 51 41 0.93 ± 0.47 0.58 ± 0.057

Aspergillus fumigatus 3.5\(^{a}\) 3.7\(^{d}\) 5.5 ± 0.45 5.2 ± 0.618 39 42 0.60 ± 0.051 0.57 ± 0.068

Aspergillus aculeatus 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\(^{d}\) 1.2 ± 0.028 5.2 ± 0.057 86 42 0.13 ± 0.002 0.5 ± 0.020
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.

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