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STUDIES ON *IN VITRO* ANTIFUNGAL ACTIVITY OF *FOENICULUM VULGARE* MILL. AGAINST SPOILAGE FUNGI

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ABSTRACT
Foeniculum vulgare Mill. commonly known as fennel, belongs to family Apiaceae. The aqueous and alcoholic seed extracts of *Foeniculum vulgare* Mill. were evaluated for their antifungal activity against - *Alternaria alternata, Mucor rouxii* and *Aspergillus flavus.* The method used for determination of antifungal activity of both aqueous and alcoholic seed extracts was agar well diffusion method. The aqueous extract of fennel seeds was found to be very effective against *Alternaria alternata.* The maximum zone (32.33 ± 2.52mm) of growth inhibition was obtained against this food spoilage fungus. Whereas the alcoholic seed extract of fennel was found to be adequately effective against Mucor *rouxii* with growth inhibition zone diameter of 2.87 ± 1.16mm. Both the extracts were completely ineffective against *Aspergillus flavus* .The results obtained from this study may contribute to the development of environmentally safer alternatives to protect the spoilage of food products from pathogenic and lipolytic fungi.

KEYWORDS: antifungal activity, seed extract, *Foeniculum vulgare, Alternaria alternata, Mucor rouxii,* spoilage fungi.

INTRODUCTION

Foeniculum vulgare Mill. commonly known as fennel belongs to family Apiaceae. It is an indigenous herb of the Mediterranean Sea shores but is also present on the dry soils near the sea coast and on the river banks (Rather *et al.,* 2012). The chemical constituents from the fennel include essential oil, fatty acid, phenylpropanoids, monoterpenids, sesquiterpenes, coumarins. It also contains triterpenoids, tannins, flavonoids, cardiac glycosides, saponins, and other types of compounds (He and Huang, 2011). Fennel is used as a constituent of the various cosmetic and pharmaceutical products (Piccaglia and Marotti, 2001).According to Anand *et al.*, 2008 fennel seed possess anticancer property. Essential oil of fennel has been known to possess diuretic, anti inflammatory, antimicrobial activity (Abed, 2007) analgesic and antioxidant activities (Choi and Hwang, 2004). Many species of fungi are responsible for the contamination of the crops before harvest or during storage by the production of aflatoxin (Yu *et al.,* 2004). There are strategies available for the prevention of fungal growth, mycotoxin production and food contamination. These strategies generally include physical, chemical and biological treatments and also require sophisticated equipments and expensive reagents (Reddy *et al.,* 2010). This has led to the increased need for searching new natural products which may act as antifungal agents so that chemical methods of preservation can be avoided (Soliman and Badea, 2002, Irkin and Korukluoglu, 2007). Numbers of reports showed efficacy of essential oil of fennel as antimicrobial agent (Ozcan *et al.,* 2006, Deans *et al.,* 1990, Caccioni *et al.,* 1998, Soylu *et al.,* 2006, Patra *et al., 2002* and Muckenstrum *et al., 1997).* However, very scanty data is found on *in vitro* antifungal activity of crude seed extract of fennel. So the objective of our study was focused on evaluation of the antifungal potential of the aqueous and alcoholic seed extracts of *Foeniculum vulgare* Mill. against the most common spoilage fungi- *Alternaria alternata, Mucor rouxii* and *Aspergillus flavus.*

MATERIALS AND METHODS-

Source and preparation of fennel seed extracts-

The seeds of *Foeniculum vulgare* Mill. (Fennel) were purchased from the Grain market, Sector -26, Chandigarh. They were surface sterilized using teepol detergent for 5 to 7 minutes followed by washing with distilled water two to three times to remove the traces of the detergent. The fennel seeds were then dried and were ground using a mixer to form a fine powder.

Preparation of aqueous and alcoholic extracts of fennel seeds

For the preparation of alcoholic fennel seed extract, 5gm fennel seed powder was soaked in 50ml of ethanol overnight whereas for the preparation of the aqueous seed extract the same amount of fennel seed powder was soaked in 50ml double distilled water overnight. The extracts were centrifuged at 7000rpm for 20 minutes. The supernatant of each extract was then filtered separately using Whatman No.1 filter paper. The alcoholic and aqueous extracts of fennel seeds were stored at 4°C till further use.

Antifungal Activity

Test microorganism

The test fungi were isolated from the rotten grapes, orange and tomato. The identification of the fungus cultures was done by adopting standard methods (Clark,1981) and the pure cultures were maintained at 32°C by subsequent sub culturing on SDA medium (Sabouraud Dextrose Agar, HiMedia).

Determination of Antifungal activity of the fennel seed extracts

To determine the antifungal activity of the alcoholic and aqueous extract of fennel seeds against the test fungi the method of agar well diffusion (Perez *et al.,* 1990) was used. 25µl of fungal suspension was added on the SDA media plates and was spread uniformly using a flame sterilized glass spreader. In the center of Petri plates wells of diameter approximately 10mm were made using sterile cork borer. In the wells, 100µl of fennel seed extract was added carefully to each well using a micropipette. The SDA plate with 100µl of autoclaved distilled water in the well served as control in all experiments. Petri plates were incubated at 32°C for 48-72 hours. The antifungal activities of the fennel seed extracts against the test fungi was evaluated by measuring the inhibition zone diameter (millimeter) surrounding each agar well. Minimum Inhibitory Concentration was calculated by using different concentrations of best performing plant extract i.e. 25%, 50%, 75% and 100% against most sensitive fungus. Triplicates of plates were prepared on each occasion and all experiments were repeated three times. The standard deviation was calculated using conventional methods. The results were represented as mean values ± standard deviation.

RESULTS

On the basis of various characteristics studied, the fungi isolated from the rotten fruits were identified as *Alternaria alternata*, *Mucor rouxii* and *Aspergillus flavus.* The antifungal activity of aqueous and alcoholic extracts of fennel seeds was assessed against the test fungi identified. The positive sign (+) indicates the inhibition of the fungal growth and the negative sign (-) indicates that there was no inhibition of fungal growth by the extract (Table-1).

TABLE 1: Effect of aqueous and alcoholic seed extracts on in *vitro* growth inhibition of tested fungi

S.No.	Names of the tested fungi	Aqueous seed extract	Alcoholic seed extract
1	*Alternaria alternata*	(+)	(-)
2	*Mucor rouxii*	(-)	(+)
3	*Aspergillus flavus*	(-)	(-)

TABLE 2: Antifungal activities in terms of zone of inhibition of aqueous and alcoholic seed extracts of fennel against tested fungi

S. No.	Names of the tested fungi	Zone of growth inhibition in millimeter (mm)	
		Aqueous seed extract	Alcoholic seed extract
1.	*Alternaria alternata*	32.33± 2.52	0 ± 0.00
2.	*Mucor rouxii*	0 ± 0.00	2.87 ± 1.16
3.	*Aspergillus flavus*	0 ± 0.00	0 ± 0.00

The results of the agar well diffusion assay and the measurement of MIC indicated the antifungal activity of the alcoholic and the aqueous extracts of the fennel seeds against the fungi- *Alternaria alternata, Mucor rouxii* and *Aspergillus flavus*. *Alternaria alternata* was found to be the most sensitive fungi. The aqueous seed extract of fennel had completely inhibited the growth of the fungus. The maximum zone of growth inhibition (32.33±2.52mm) of aqueous extract of fennel was obtained against *Alternaria alternata.* MIC values of aqueous extract (Table 3) showed that the minimum concentration of inhibition was 25% where the mean zone of inhibition obtained was 20.33±0.58mm, which further increased with the increase in concentration of the aqueous seed extract. The alcoholic seed extract showed appreciable inhibition of growth against *Mucor rouxii*. The diameter of the growth inhibition zone obtained was 2.87 ± 1.16mm. Whereas, both alcoholic and aqueous extracts of fennel were found to be completely ineffective against *Aspergillus flavus.*

TABLE 3: Antifungal activity in terms of MIC of aqueous seed extracts of fennel against fungus *Alternaria alternata*

S.No.	Concentration of the aqueous seed extract	Zone of growth inhibition in millimeters (mm)
1	25%	20.33 ± 0.58
2	50%	25.33 ± 2.52
3	75%	27.5 ± 0.50
4	100%	32.33 ± 2.52

DISCUSSION

Plant extracts obtained from various medicinal plants possess antimicrobial activity against many food borne, human and plant pathogens and pests (Isman, 2000, Kalemba and Kunicka, 2003, Burt 2004). Several studies have been conducted to check the antimicrobial properties of different herbs, spices, and the derivatives of these herbs and spices like essential oils, extracts and decoctions. (Farag *et al.,* 1989, Bowers *et al.,* 2000, Dorman *et al.,* 2000). Many plant extracts obtained from medicinal plants have been used for food preservation and for medicinal purposes due to their antimicrobial effects (Cowan 1999, Lee *et al.,* 2007, Tassou *et al.,* 2000, Valero and Salmeroj, 2003). Some plants may be alternatives to currently used disease control agents since they constitute rich source of bioactive chemicals. Numbers of reports showed efficacy of essential oil of fennel as antimicrobial agent. However, a few reports are found on *in vitro* antifungal activity of crude seed extract of fennel. In the present study, we have reported the effectiveness of

aqueous and alcoholic extract of fennel against different spoilage fungi. Aqueous extract of fennel fruits contains rich phenolic compounds, hydroxyl- cinnamic acid derivatives, flavonoid glycosides and flavonoid aglycones (Parejo *et al*., 2004).The antifungal activity of fennel extract may be due to presence of these secondary metabolites (Kaur and Arora, 2009)and anethole (Ozcan and Chalchat, 2006). The mechanisms thought to be responsible for phytochemical toxicity of plant extracts to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins. In the present study, water extract of seed of fennel showed a marked inhibition of *Alternaria alternata* fungus whereas the alcoholic seed extract was ineffective against this fungus. The effectiveness of water (aqueous) extract of fennel in inhibiting this fungus species could be attributed to the fact that water is a polar solvent and the phytochemical constituents of fennel such as flavanoids are very soluble in it hence retaining most of the antimicrobial (antifungal) properties during the extraction process (Hasan *et al.*, 2005). The plant extract was completely ineffective against the other two test fungi namely *Aspergillus flavus* and *Mucor roxuii.* This varied susceptibility to the plant extract could be due to inherent physiological and morphological characteristics of species involved in the study. According to Mughal *et al.*, 1996 the aqueous leaf extract of fennel inhibits the growth of *A. alternata* and *A. brassicola*. Fennel extracts have been reported to inhibit the growth of *Curvularia lunata*, *Fusarium oxysporum* and *A. alternata* by Prabha *et al*., 2002. The alcoholic based extract was found adequately effective against *Mucor roxuii* but was completely ineffective against *Aspergillus flavus* and *Alternaria alternata.* The reduced effectiveness of alcoholic extract against test fungi could be attributed to the solubility and volatility of its phytochemical components and losses during the process of extraction especially in organic solvents. The MIC values were calculated for the aqueous extract of the seeds which showed the increase in growth inhibition of test fungi with the increase in the concentration of plant extract. The crude extract contains mixture of active and inactive compounds and MIC of less than 100µg/mL suggests strong antimicrobial activity (Webster *et al*., 2008). In this research MIC value was of 250µg/mL which suggest the good antifungal activity of aqueous seed extract against *Alternaria alternata.* The quantity and quality of these active compounds depends on the plant species, plant tissue under study and environmental factors (Demo and Oliva, 2008, Webster *et al.*, 2008).

CONCLUSIONS

The significant growth inhibitions of the test fungi by the both aqueous and alcoholic seed extracts of fennel suggest their possible use in controlling these fungi in disease causing situations and food spoilage. In particular, aqueous seed extract offer effective bioactive compounds for growth inhibition of the *Alternaria alternata*. Even at low concentrations, this extract showed strong antifungal activity. Further studies are needed to isolate the active ingredients responsible for the observed antifungal activity. Natural plant-derived fungicides may provide better alternatives to the conventional antifungal additives in foods. Furthermore, the easy means of obtaining these extracts especially using water base extraction provides an alternative to antibiotics and artificial preservatives both of which can be toxic at certain concentrations.

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