

Antifungal Potency of *Foeniculum vulgare* Seed Extract

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Abstract

Foeniculum vulgare belongs to family Apiaceae and is also called by the name of fennel. Agar disc diffusion method was used to determine the antifungal activity of its seed extracts in water and methanol against a number of pathogenic fungi. Methanolic extract of fennel seeds was less effective against test fungi as compared to its aqueous extract. However in case of *Candida* species, both types of extracts were found to be effective.

Keywords: Apiaceae; Fennel; Seed extract

Introduction

Foeniculum vulgare is a perennial herb that belongs to the family Apiaceae. Traditionally, it is used as an antiseptic, carminative, digestive, diuretic, and expectorant agent. *Foeniculum vulgare* is also known as fennel and is mostly found in southern Europe and the Mediterranean region. Nowadays, it occupies a large area in the temperate and tropical regions of the world [1]. *Foeniculum vulgare* plants have hollow branches with an average height of 2.5 m. Its leaves are finely dissected with thread like segments that are about 0.5 mm in width. The flowers are positioned at the terminals carrying seeds that are about 4-10 mm in length [2]. *Foeniculum vulgare* leaves and its fruit are mainly used as ingredients in cosmetics and as flavoring substances for fish and meat to give them a strong aroma and taste [3]. Its essential oil, which is produced by steam distillation of dried fruits, is used for flavoring purposes in Western countries [4]. *Foeniculum vulgare* seed and bark extracts have been reported to exhibit antifungal effect against *Candida* species by inhibiting mycelial growth and germination of *Sclerotinia sclerotiorum* [5,6]. Thus could be used as effective bio fungicide against phytopathogenic fungi [7]. Besides fennel seed possess anticancer property anti-inflammatory, antimicrobial activity and antioxidant activities [5,8,9]. *Foeniculum vulgare* seed extract has antifungal activity against various fungal species such as *Candida albicans*, species of *Aspergillus* and dermatophytes [10]. However, very little information is available on the antimicrobial activity of fennel seed extract in vitro. So the purpose of this research work was to demonstrate and evaluate the antifungal potency of *Foeniculum vulgare* seed extracts against different pathogenic fungi.

Materials and Methods

Foeniculum vulgare seed extract preparation

Foeniculum vulgare seeds were bought from the local market, Satellite town Quetta. Seeds were washed with distilled water twice and then were dried at 40°C. *Foeniculum vulgare* seeds were milled into a fine powder using a grinder.

Foeniculum vulgare seed aqueous and alcoholic extract preparation

For the formation of alcoholic and aqueous seed extract, 5 g of milled seed powder was weighed accurately and suspended in 50 ml of methanol and distilled water overnight, separately (Figure 1). The flasks were shaken for three hours and then filtered through Whatman No.1 filter paper. The filtrate was centrifuged at 5000 rpm for 5 to 10 min. The final concentration of each extracts was fixed to about 100 mg/ml.

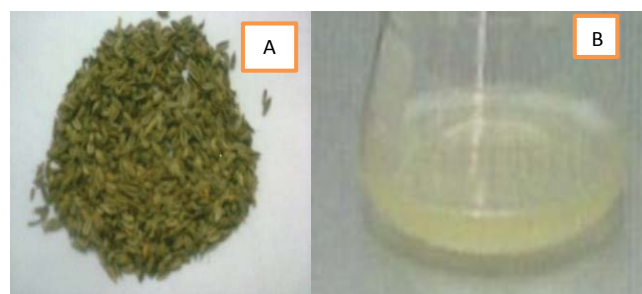


Figure 1: *Foeniculum vulgare* seeds extract. A: Seeds, B: Extract.

Disc diffusion method

Antifungal activity of aqueous and methanolic extract was individually tested against a number of pathogenic fungi using disc diffusion method. Already prepared cultures of test fungi were maintained at 32°C by subsequent subculturing. Agar surface (YM agar) was inoculated with *Candida glabrata*, *Aspergillus flavus*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *Alternaria alternata* and *Mucor rouxii*.

Sterile discs preparation

1cm sized sterilized discs were impregnated with different concentration of alcoholic and aqueous seed extract (20 µL, 40 µL, 60 µL and 80 µL) and placed on agar surface, incubated for 48 h at 37°C (Figure 2). Presence or absence of inhibition zone around the impregnated discs indicated the antifungal property of the extracts. YM plates with discs impregnated with different concentrations of autoclaved distilled water was taken as control in all experiments.

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Figure 2: Disc diffusion method containing alcoholic and aqueous seed extract impregnated discs.

S. No.	Names	Aqueous extract	Methanolic extract
		Inhibition zone	Inhibition zone
1	<i>Candida glabrata</i>	+	+
2	<i>Candida krusei</i>	-	-
3	<i>Candida albicans</i>	+	+
4	<i>Candida parapsilosis</i>	+	-
5	<i>Candida tropicalis</i>	+	+
6	<i>Aspergillus flavus</i>	-	-
7	<i>Alternaria alternata</i>	+	-
8	<i>Mucor rouxii</i>	-	+

(+) indicates the inhibition of the fungal growth
 (-) indicates no inhibition of fungal growth

Table 1: Observed antifungal activity based on the presence or absence of inhibition zone.

Results

Foeniculum vulgare seed extracts were evaluated for their antifungal (Table 1).

Discussion

Several studies revealed that essential oil extracted from fennel seed act as effective antimicrobial agent [11]. But reported research related to determine the antifungal activity of fennel seed extract is scanty. So in this particular study, we have determined the antifungal activity of fennel seed extracts in water and methanol. The anti-fungal activity of fennel seed aqueous extract is due to the presence of secondary metabolites such as flavonoid, phenols, aglycones, glycosides, hydroxyl-cinnamic acid derivatives and anethole [12,13]. The phytochemical toxicity effect of fennel seed extracts on pathogens includes protein inhibition by oxidation involving sulfhydryl groups or by means of non-specific interactions. The obtained results clearly indicates that aqueous fennel seed extract showed effective inhibition of *Alternaria alternata* fungus while on the other hand its methanolic extract was ineffective against this fungus. The possible reason for this is due to the polar nature of the water, the chemical constituents of the extract such as flavonoids were very soluble in water. Therefore, retained its antimicrobial (antifungal) properties during the extraction process [14]. But in case of *Aspergillus flavus* and *Mucor rouxii* both the extracts were completely ineffective as indicated by the absence of inhibition zone. This may be due to intrinsic biological and morphological features of species used in the study. However it is reported before that the aqueous leaf extract of fennel is a

good inhibitor of *A. alternata*, *A. brassicola* [15], *Curvularia lunata* and *Fusarium oxysporum* [16]. Methanolic extract inhibited *Mucor rouxii* growth but no inhibition zone was observed in agar plates inoculated with *Aspergillus flavus* and *Alternaria alternata*. The supporting reason for this is the decreased solubility and volatility of its chemical constituents during organic solvent based extraction process. But the antimicrobial activity of these active compounds are greatly influenced and affected by the environmental conditions under which they are extracted and also the parts of the plant from where they are obtained [17,18].

Conclusion

Based on the presence of inhibition zone surrounding the extracts impregnated disc we can conclude that fennel seed extracts could be used in the preparation of an effective antifungal agent with a broad activity spectrum against a number of disease causing fungi. However further studies need to be conducted to isolate the active bio compound from the extracts to understand its chemical composition responsible for the antifungal activity. Furthermore using water base extraction methods provides an easy and cheapest way to obtain extracts with potential antifungal activity.

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