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Anti-microbial properties of fennel (*Foeniculum vulgare* Mill.) seed extract

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Abstract

Foeniculum vulgare has been used as an ethnic remedy for the cure of numerous infectious disorders of bacterial, fungal, viral, and mycobacterial origin. Several studies have been carried out in the past validating its antimicrobial, anti-mycobacterial and antiviral potential. Present study was conducted to validating anti-bacterial and anti-fungal properties of methanolic and hexane seed extract of five fennel genotypes commonly grown in India. The study showed genotypic variation in anti-microbial effect of both the extracts. The findings indicated significant anti-microbial activity in seed extract. *Foeniculum vulgare* has potential to be used as a good source of traditional medicine and it provides a noteworthy basis in pharmaceutical biology for the development/formulation of new drugs and future clinical uses.

Keywords: Anti-bacterial, anti-fungal, *foeniculum vulgare*, pharmacology, seed extract.

1. Introduction

Fennel (*Foeniculum vulgare* Mill) is an annual herbaceous plant belongs to the family *Apiaceae*. It is widely cultivated throughout the temperate and tropical regions of the world. Fennel herb is being used as a medicinal and economic plant in Asian countries. Survey of published literature revealed that fennel herb and seeds effectively control numerous infectious disorders of bacterial, fungal, viral, mycobacterium, and protozoal origin as well as used antispasmodic, diuretic, anti-inflammatory, analgesic, secretomotor, secretolytic, galactagogue, eye lotion, and antioxidant remedy in Europe and Mediterranean areas [1, 2, 3, 4]. Fennel seeds essential oil is used as flavouring agents in food products such as liqueurs, bread, cheese, pickles and pastries [5] and an ingredient of cosmetics and pharmaceutical products [6]. In India, fennel is cultivated in semi-arid regions of Gujarat and Rajasthan states with an area of 46760 hectare and production of 78570 tonnes during 2015-16 (E) (Spice Board, India 2017). Approximately 20% of the fennel production has been exported to Middle East, Europe and USA. Fennel is one of the important crops having the potential to be used as medicinal and industrial purpose. In present investigation seeds of five popular fennel varieties have been taken and their crude extracts in two different solvents have been evaluated for its antibacterial and anti-fungal properties.

2. Materials and methods

Plant material

Mature seeds of fennel varieties Ajmer Fennel-1 (AF-1), Gujarat Fennel-2 (GF-2), Rajasthan Fennel-101 (RF-101), Hisar swarup and Rajendra Sourabha were taken from gene bank of ICAR-NRCSS, Ajmer, India from fresh harvest. Pure and healthy seeds of each variety were used for preparation of crude seed extract in different solvents.

Bacterial and fungal strains

Bacterial strains gram positive (*Staphylococcus aureus*, *Streptococcus Pyogenes*) and gram negative (*Escherichia Coli*, *Pseudomonas aeruginosa*) and fungal strains (*Candida albicans*, *Aspergillus clavatus*) were chosen based on their clinical and pharmacological importance. The bacterial microorganisms were cultured on nutrient agar by using spread plate technique and fungal stock cultures were incubated for 24 hours at 37°C on potato dextrose agar (PDA) medium, following low temperature storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the fungal stains were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

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Chemicals and reagents

All chemicals and reagents (analytical HPLC grade) used in present study were procured from Merck Co. (Germany) and Sigma-Aldrich (USA).

Determination of Zone of inhibition method

In vitro anti-bacterial and antifungal activities of fennel crude seed extract in methanol and hexane were examined against two gram positive and two gram negative pathogenic bacteria and two fungi by the agar disk diffusion method. Each sample were dissolved in dimethyl sulphoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. The dilutions (25µg/ml) of sample and standard drugs (5, 25 and 50 µg/ml) were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using a cefixime and griseofulvin as standard drugs respectively. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks. The values <8 mm were considered as not active. Agar diffusion test (Mueller-Hinton test) is a test which uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. Known quantities of bacteria are grown on agar plates in the presence of thin wafers containing relevant antibiotics. If the bacteria are susceptible to particular antibiotics, an area of clearing surrounds the wafer where bacteria are not capable of growing called a zone of inhibition.

Cefixime was used as standard drug against the bacterial infection. Cefixime 25 µg/ml was used and the zone of inhibition in mm was consider as the control (100%), 32mm in *Staphylococcus aureus*, 35 mm *Streptococcus pyogenes*, 37mm *E. Coli* and 37mm *P. aeruginosa*. Similar concentration of fennel genotypes were used to detect the antibacterial activity over the gram positive and gram negative bacteria.

3. Results and discussion

Anti-bacterial Potency of fennel genotypes in different solvent extracts

In gram positive bacterial group *Staphylococcus aureus* had

minimum zone of inhibition in AF-1 (16 mm) whereas RF-101 showed maximum zone of inhibition (19 mm), followed by GF-2, Rajendra Saurbha (18 mm) and Hisar Swarup (17 mm). Next bacteria under this group *Streptococcus pyogenes* had minimum zone of inhibition in the methanol extracts of genotype RF-101 and Hisar Swarup (19 mm) whereas AF-1, GF-2 and Rajendra Saurbhs showed equal zone of inhibition (21 mm). This was highest in terms of percentage zone inhibition (76%) within methanol extracts of all five genotypes in both the bacterial groups (Table 1). In case of seed extract in hexane solvent gram positive bacterial group *Staphylococcus aureus* had minimum zone of inhibition in Hisar Sawroop In (17mm) whereas AF-1 showed maximum zone of inhibition (21 mm), followed by RF-101 (20 mm), Rajendra Saurbha (19 mm) and GF-2 (18 mm). Another bacteria under this group *Streptococcus pyogenes* had minimum zone of inhibition in the hexane extracts of genotype AF-1 (14 mm) and maximum zone of inhibition in RF-101 (19 mm) whereas Hisar Swarup and Rajendra Saurbhs showed equal zone of inhibition (18 mm) and GF-2 showed 17 mm. Seed extract of genotype AF-1 was most effective in terms of percentage (76%) in both the bacterial groups (Table 1).

In Gram Negative bacterial group *E. coli* showed maximum zone of inhibition in RF-101 (17 mm) followed by AF-1 and Rajendra Saurbha (16 mm), Hisar Sawarup (15 mm) and GF-2 with minimum (14 mm) zone of inhibition. Next bacteria under this group *P. aeruginosa* had minimum zone of inhibition in the methanol extracts of genotype Hisar Sawroop (14 mm) followed by Rajendra Saurbha (16 mm), RF-101 (17 mm) and GF-2 (18 mm). Highest zone of inhibition was observed in AF-1 (19 mm) (Table 1). In case of seed extract in hexane solvent gram negative *E. coli* showed the maximum zone of inhibition in RF-101 (19 mm) followed by AF-1 (18 mm), Hisar Sawarup (16 mm) and GF-2 (15 mm) and minimum zone of inhibition in Rajendra Saurbha (13 mm). Next bacteria under this group *P. aeruginosa* had minimum zone of inhibition in genotype GF-2 (15 mm) followed by Hisar Sawarup (16 mm), AF-1 and Rajendra Saurbha showed equal zone of inhibition (19 mm). Highest zone of inhibition was observed in RF-101 (21 mm) (Table 1). When compared both the extract, hexane seed extract showed more anti-bacterial activity than methanol seed extract.

Table 1: Antibacterial activity of seed extracts of fennel genotypes (zone of inhibition in mm)

Seed extract (25 µg/ml)	Zone of inhibition (mm)							
	<i>S. aureus</i>		<i>S. pyogenes</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	Methanol	Hexane	Methanol	Hexane	Methanol	Hexane	Methanol	Hexane
Cefixime	32	32	35	35	37	37	37	37
AF-1	16 (58)	21 (76)	21 (76)	14 (52)	16 (58)	18 (66)	19 (69)	19 (69)
GF-2	18 (66)	18 (66)	21 (76)	17 (62)	14 (52)	15 (54)	18 (66)	15 (54)
H.Swarop	17 (62)	17 (62)	19 (69)	18 (66)	15 (54)	16 (58)	14 (52)	16 (58)
R. Saurabha	18 (66)	19 (69)	21 (76)	18 (66)	16 (58)	13 (50)	16 (58)	19 (69)
RF-101	19 (69)	20 (73)	19 (69)	19 (69)	17 (62)	19 (69)	17 (62)	21 (76)

Value in parenthesis is zone inhibition in percentage

Anti-fungal Potency of fennel genotypes in different solvent extracts

Antifungal activity of methanol and hexane extract of fennel was analysed using Griseofulvin as standard drug against the fungal infection. Griseofulvin 25 µg/ml was used and the zone of inhibition in mm was consider as the control (100%),

which was 46 mm in *Candida albicans*, 45 mm *Aspergillus clavatus*. Similar concentration of fennel genotypes was used to detect the antifungal activity.

When methanol seed extract of each genotypes was used as drug, fungal strain *Candida albicans* had minimum zone of inhibition in GF-2 and RF-101 (21 mm) whereas AF-1 and

Hisar Swarup showed maximum zone of inhibition (23 mm) followed by Rajendra Saurabh (22 mm) (Table 2). *Aspergillus clavatus* had minimum zone of inhibition in the methanol extracts of genotype GF-2 (16 mm) and maximum zone of inhibition in genotype RF-101 (25 mm) followed by Rajendra Saurabh (23 mm), Hisar Swarup (21 mm) and AF-1 (17 mm). RF-101 was the highest in terms of percent inhibition (78%) in all five genotypes on fungal strain *Aspergillus clavatus* (Table 2).

In place of methanol, hexane seed extract was used to compare both extract. Fungal strain *Candida albicans* had minimum zone of inhibition in RF-101 (19 mm) whereas AF-

1 and Rajendra Saurabh showed maximum zone of inhibition (21 mm), genotype GF-2 and Hisar Swarup showed similar zone of inhibition (20 mm) (Table 2). Another strain *Aspergillus clavatus* had minimum zone of inhibition in genotype AF-1 (17 mm) and maximum in genotype GF-2 (21 mm) followed by RF-101 (19 mm). Hisar Swarup and Rajendra Saurabh showed equal zone of inhibition (18 mm). AF-1 was the highest in terms of percentage (65%) in all five genotypes in both fungal strains (Table 2). Anti-fungal activity was more in methanol seed extract as compared to hexane.

Table 2: Antifungal activity of seed extracts of fennel genotypes (zone of inhibition in mm)

Seed extract (25 µg/ml) and standard fungicide	Zone of inhibition in mm			
	<i>Candida albicans</i>		<i>Aspergillus clavatus</i>	
	Methanol	Hexane	Methanol	Hexane
Griseofulvin	46	46	45	45
AF-1	23 (72)	21 (65)	17 (54)	17 (54)
GF-2	21 (65)	20 (62)	16 (51)	21 (65)
H. Swarop	23 (72)	20 (62)	21 (65)	18 (57)
R.Saurabh	22 (68)	21 (65)	23 (72)	18 (57)
RF- 101	21 (65)	19 (60)	25 (78)	19 (60)

Value in parenthesis is zone inhibition in percentage

There are many reports establishing the anti-microbial properties of fennel herbs and seeds. An aqueous extract of seed sample inhibited the growth of *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri*, and *Bacillus cereus* [1, 2, 3, 4]. According to the results reported by [7] essential oil of *F. vulgare* had significant antimicrobial activities against some of microorganisms as compared to the methanolic and ethanolic extracts [8] investigated antimicrobial effect of the methanol, ethanol, diethyl ether, and hexane extracts of seed of *F. vulgare* against two species of Gram negative bacteria (*Escherichia coli* and *Salmonella typhi*), two species of Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*), one species of yeast (*Candida albicans*), and one species of mold (*Aspergillus flavus*). In agreement with present report, the methanolic extract showed more effective antimicrobial activity than the other extracts [9] investigated the antibacterial effect of the crude, chloroform, and methanol extract of leaves and flowers of *F. Vulgare* along with *Raphanus sativus* and *Brassica nigrum* against *Escherichia coli* and *Staphylococcus aureus*. Methanol extract of flower of *F. vulgare* showed significant activity against *Escherichia coli*, whereas crude and chloroform extracts failed to exhibit antimicrobial activity against *Staphylococcus aureus*. Similar to present results among different tested bacterial strains, the methanolic fruit extract of *F. vulgare* inhibited the growth of *Staphylococcus aureus* and *Bacillus pumilus* with 11.27 and 12.67mm zone of inhibition, respectively [4].

Essential oil of *F. vulgare* showed appreciable antifungal activity against strains of pathogenic fungi, namely, *Aspergillus niger*, *Fusarium solani*, and *Rhizopus solani* [10]. Dichloromethane extracts and essential oils from *F. vulgare* showed antifungal activity against *Candida albicans*. It could be a potential candidate for a new antifungal agent for candidiasis and other fungal diseases [11]. In an *in vitro* study, aqueous and alcoholic seed extracts of *F. vulgare* exhibited inhibitory effect against *Alternaria alternata*, *Mucor rouxii*, and *Aspergillus flavus* [12]. Interestingly, aqueous seed extract

of *F. vulgare* showed strongest antifungal activity as compared to reference fungicidal agent, that is, griseofulvin [13].

All of the above mentioned studies were carried out on the crude extracts and it is difficult to pinpoint the active antimicrobial metabolite. A phenylpropanoid derivative called dillapional, characterized from *F. vulgare* stem and a coumarin derivative, scopoletin was found to be marginally antimicrobial agent [14]. The characterization of seven different types of oxygenated monoterpenes, from methylene chloride crude extract of *F. Vulgare* [15] suggested that the crude extract containing monoterpenes could be a new medicinal resource for antibacterial agents.

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