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Comparative *in vitro* antimicrobial potential of *Foeniculum vulgare* Mill fruits extracts

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ABSTRACT

Aim of the study: The present study was focussed on comparative antimicrobial activity of ethanolic and aqueous extracts of *Foeniculum vulgare* Mill fruits which were obtained from three different regions of India such as FR-1 from Uttrapradesh, FR-2 from Gujrat and FR-3 from West Bengal. **Materials & methods:** The antimicrobial study was done by using the Agar well diffusion and broth dilution method against bacteria (*E.coli, S. aureus, P. aeruginosa*) and fungi (*A. Niger, C. albicans*). The diameter of zone of inhibition was measured against all tested microbes and minimum inhibitory concentration was calculated by using tube dilution method as tested against the turbidity in test tube. **Results:** The result showed that aqueous extract prepared from the sample (FR-3) was found to be most effective against bacteria *B.substilis* and *S.aureus* and against fungi *A. niger* while the ethanolic extract of sample (FR-2) was most suspectible against bacteria *E.coli* and fungi *C.albicans*. **Conclusions:** With a wide spectrum of inhibition against tested microbes, extracts of *Foeniculum vulgare* is worthy of further investigation as a natural wide spectrum antibacterial agent in the treatment of infectious disease. The variation observed in the activity of the extracts prepared from different samples of *Foeniculum vulgare* may be due to one or more reasons such as difference in geographical region, difference in the amount of active chemical constituents, difference in method of collection, preparation, storage and difference in the seasons of plant collected.

KEYWORDS: Antimicrobial, *Foeniculum vulgare*, Umbelliferae, Agar well Diffusion, MIC

INTRODUCTION

Foeniculum vulgare Mill commonly known as Fennel, belonging to family Umbelliferae is a small group of annual, biennial or perennial herb[1]. Traditionally fruits are used as carminative, spasmolytic, hypotensive[2] bitter, sweet, acrid, emollient, refrigerant, alexipharmic, expectorant, haematinic[3,4,5]. The plants available in market from different regions is likely to vary in quality and therapeutic activity due to difference in various geographical regions, difference in method of cultivation, collection, storage and seasons of plants collected[6]. The purpose of the present study is to compare the *in vitro* antimicrobial activity of aqueous and ethanolic extracts of *Foeniculum vulgare* Mill fruits collected from three different regions of India by using Agar Well diffusion method[7,8] and minimum inhibitory concentration (MIC) against various bacterial and fungal strains as mentioned below.

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MATERIALS AND METHODS

Plant material

The diseased free fruits of *Foeniculum vulgare* Mill were collected from three different regions of India such as FR-1 from Uttar Pradesh, FR-2 from Gujarat and FR-3 from West Bengal and the voucher specimens submitted at the Pharmaceutical Department of Guru Jambheshwar University, Hisar.

Extraction Procedure of Plant Material

For the preparation of ethanolic and aqueous extracts, 25g of air dried coarsely powdered fruits of *Foeniculum vulgare* Mill were extracted with 100 ml of 90% v/v ethanol and distilled water respectively by using the soxhlet apparatus. The extracts were filtered through Whatman filter paper 42. The filtrates were evaporated to dryness on the water bath. The prepared extracts were kept in desiccators and stored properly in refrigerator before use for antimicrobial activity.

Microbial Strains Used

The bacterial strains *Escherichia coli* (NCIM 2065), *Staphylococcus aureus* (NCIM 2901), *Bacillus substilis* (NCIM 2106) used for the proposed antibacterial study were obtained from the National Chemical

Laboratory (NCL), Pune, Maharastra, India and the fungal strains *Aspergillus niger* (NCIM 590), *Candida albicans* (MTCC 227) for the antifungal activity were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India.

Culture Media used

Nutrient Agar medium and Sabouraud's dextrose agar medium obtained from the HiMedia Bombay were used for evaluate the antibacterial and antifungal activity respectively of the prepared extracts[8, 9]. The specified amount of culture media was dissolved in specified amount of distilled water and then heated to boiling for about 1 minute and thereafter the media was sterilized by using autoclave at 15 lb/square inch pressure at 121ºC for 15-20 min.

Preparation of Standard and Test Compounds Solution

For evaluate the antimicrobial activity by using Agar well diffusion method, concentration of 1000 µg/ml of Standard drugs (Ampicillin trihydrate for the bacterial assay and clotrimazole for the assay of fungi) and 2000 µg/ml of the test compounds were prepared by dissolving in DMSO (Dimethyl Sulphoxide) in small volumetric flasks.

Antimicrobial activity

Aseptically 0.2 ml of each seeded broth containing 10^6-10^7 cfu/ml of test organism was inoculated onto sterilized nutrient agar media containing in the sterile petriplates and spreaded uniformly with the help of glass spreader. Then plates were allowed to solidify. 4 wells of 6 mm internal diameter were made by punching with sterile stainless steel cork borer and numbered as a, b, c and d. To the first three cavities of each petriplates test samples of 2000 µg/ml concentration were added aseptically while in the fourth cavity pure solvent DMSO was added which was taken as a normal control. Three replicates were used for each drug extract. Standard solution of 1000 µg/ml of ampicillin trihydrate for bacterial assay and clotrimazole for fungal assay were introduced in well taken as drug control. The plates were allowed to stand for 1 h for diffusion of solution and then incubated in incubator at temperature 37ºC ± 1ºC for 24 hrs for bacteria and 72 hrs for fungus *Candida albicans* and at 25ºC± 1ºC for fungal strain *Aspergillus niger* for a period of 7 days**.** The zone of inhibition formed around the cups in the form of transparent area after incubation was measured in millimeters.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of test compounds was determined by using the turbidity method or tube dilution method [9, 10]. Eight assay tubes containing the 1 ml of sterile nutrient broth medium for bacterial strains and sabouraud dextrose broth medium for the fungal strains were used for screening minimum inhibitory concentration. These test tubes were labelled 1-8. Using a sterile graduated pipette 1 ml of each plant extract being examined was pipetted out into the test tube labelled as 1, mixed well using a fresh pipette and then 1 ml was pipetted out into the test tube labelled as 2, mixed well and further I ml was pipetted out into the test tube labelled as 3 until the last test tube labelled as 8. From the eighth test tube 1 ml was then pipetted out and discarded to get the concentration 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125µg/ml respectively from test tube 1 to 8. Then 0.2 ml of microbial suspension of each strain containing 10^6-10^7 cfu/ml of test organism was added aseptically. The positive control was also maintained by adding the standard drug while the tubes containing growth medium with inoculums and solvent served as normal control. The rack of assay tubes were incubated at 37ºC ± 1ºC for 24 hrs for bacteria and for 72 hrs for the fungus *Candida albicans* and at 25 ºC± 1ºC for fungal strain *Aspergillus niger* for a period of 7 days**.** After incubation period, the assay tubes were observed for any deposit or turbidity in the solution, shaken to suspend bacteria and fungi that might have been settled down. The lowest concentration of the extracts and the standard drug that caused apparently a complete inhibition of growth of organism were taken as minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSIONS

Results of Agar well diffusion method

The results showed that both ethanolic and aqueous extracts of each sample showed significant antimicrobial activity in terms of zone of inhibition against all the tested microbial strains used for the proposed study as shown in Table 1. Aqueous extract prepared from the sample (FR-3) was found to be most effective against bacteria *B.substilis* and *S.aureus* and against fungi *A.niger* while the ethanolic extract of sample (FR-2) was most suspectible against bacteria *E.coli* and fungi *C.albicans*. The concentration of 1000 µg/ml of standard drugs ampicillin trihydrate for the bacterial assay showed maximum zone of inhibition of 27 mm against *B. substilis* followed by the *S. aureus* with zone of inhibition of 12 mm while the standard drug clotrimazole used for assay of fungi showed zone of inhibition of 42 mm against the tested fungi.

Table 1- Zone of inhibition of extracts of *Foeniculum vulgare* Mill. against microbial strains

Sample Name	Conc.(µg/ml)	Zone of inhibition (mm) E.coli	B.substilis	S.aureus	C.albicans	A. niger
FR-1 Ethanolic	2000	13	13	12	13	12
FR-2 Ethanolic	2000	13	13	12.5	13	12.5
FR-3 Ethanolic	2000	14	12	12	11	13
FR-1 Aqueous	2000	12.5	12	12.5	11	12
FR-2 Aqueous	2000	11	13	12	13	12
FR-3 Aqueous	2000	12	13.5	15	11	13
Standard	1000	21	27	24	42	42

Table 2-Serial dilution of *Foeniculum vulgare* Mill. extracts against microbial strains

Extract	Serial dilution (µg/ml)							
	1000	500	250	125	62.5	31.25	15.625	7.8125
Escherichia coli								
FR-1 Ethanolic	–	–	–	–	–	+	+	+
FR-2 Ethanolic	–	–	–	–	–	+	+	+
FR-3 Ethanolic	–	–	–	–	–	+	+	+
FR-1 Aqueous	–	–	–	–	–	+	+	+
FR-2 Aqueous	–	–	–	–	–	+	+	+
FR-3 Aqueous	–	–	–	–	–	+	+	+
Stahylococcus aureus								
FR-1 Ethanolic	–	–	–	–	+	+	+	+
FR-2 Ethanolic	–	–	–	–	+	+	+	+
FR-3 Ethanolic	–	–	–	–	+	+	+	+
FR-1 Aqueous	–	–	–	–	+	+	+	+
FR-2 Aqueous	–	–	–	–	+	+	+	+
FR-3 Aqueous	–	–	–	–	+	+	+	+
Bacillus substilis								
FR-1 Ethanolic	–	–	–	–	+	+	+	+
FR-2 Ethanolic	–	–	–	–	+	+	+	+
FR-3 Ethanolic	–	–	–	–	+	+	+	+
FR-1 Aqueous	–	–	–	–	+	+	+	+
FR-2 Aqueous	–	–	–	–	+	+	+	+
FR-3 Aqueous	–	–	–	–	+	+	+	+
Aspergillus niger								
FR-1 Ethanolic	–	–	–	–	+	+	+	+
FR-2 Ethanolic	–	–	–	–	+	+	+	+
FR-3 Ethanolic	–	–	–	–	+	+	+	+
FR-1 Aqueous	–	–	–	–	+	+	+	+
FR-2 Aqueous	–	–	–	–	+	+	+	+
FR-3 Aqueous	–	–	–	–	+	+	+	+
Candida albicans								
FR-1 Ethanolic	–	–	–	+	+	+	+	+
FR-2 Ethanolic	–	–	–	+	+	+	+	+
FR-3 Ethanolic	–	–	–	+	+	+	+	+
FR-1 Aqueous	–	–	–	+	+	+	+	+
FR-2 Aqueous	–	–	–	+	+	+	+	+
FR-3 Aqueous	–	–	–	+	+	+	+	+

– = No Growth; + = Growth.

Table 3- MIC of *Foeniculum vulagre* Mill extracts against microbial strains

Name of Organism	Minimum inhibitory concentration (MIC) µg/ml							
	FR-1 Eth	FR-2 Eth	FR-3 Eth	FR-1 Aq	FR-2 Aq	FR-3 Aq	DMSO	Ampicillin trihydrate / clotrimazole
E.coli	62.5	62.5	62.5	62.5	62.5	62.5	0	0.156
B. substilis	125	125	125	125	125	125	0	0.156
S. aureus	125	125	125	125	125	125	0	0.156
C. albicans	250	250	250	250	250	250	0	0.156
A. niger	125	125	125	125	125	125	0	0.156

Results of Broth dilution method

Results showed the MIC values obtained by all tested extracts against bacterial and fungal strains respectively (Table 2). All aqueous and ethanolic extracts showed maximum suspectibility against *E.coli* while minimum activity was showed against *Candida albicans* (Table 3). All tested samples showed MIC 62.5 µg/ml against E.coli and against *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger* MIC value was found to be same which was found to be 125 µg/ml as

compared to standard drug Clotrimazole (MIC value=0.156 µg/ml).

CONCLUSION

From this comparative study it is of utmost importance to ensure the quality, uniformity and consistency of crude drugs used for the further uses. Plants collected from different geographical origin may lead to difference in active chemical constituents due to one or more reasons such as difference in method of collection, preparation, storage and difference in the seasons of plant collected which further may responsiple for variation in the proposed biological activity. The results of comparative antimicrobial study of aqueous and methanolic extracts of *Foeniculum vulgare* also showed a significant inhibition against tested microbes, hence extracts of *Foeniculum vulgare* is worthy of further investigation as a natural wide spectrum antibacterial agent in the treatment of infectious disease.

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