ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

MICROBIOLOGY

ANTIBACTERIAL SCREENING ON *FOENICULUM VULGARE* MILL.

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ABSTRACT

Ethanol, methanol and aqueous extracts of *Foeniculum vulgare* Mill. seeds were investigated for *in vitro* antibacterial screening by Agar well and Disc diffusion method against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterobacter aerogeus, Salmonella typhi, Bacillus cereus* and *Staphylococcus aureus. In Both* agarwell and disc diffusion methods, the highest inhibition zone was found in aqueous extracts. It showed greater activity against the maximum tested bacteria followed by ethanol and methanol extract. The preliminary results of this study indicate that the aqueous seed extracts have potentials for antibacterial activity.

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

KEYWORDS

Foeniculum vulgare, antibacterial screening, seed extracts, agar well and disc diffusion methods, inhibition zone.

INTRODUTION

Medicinal plants are important sources of potentially useful new compounds for the development of chemotherapeutic agents. The first step towards this goal is screening of plants used in popular medicine. Thus, antibacterial research is geared towards the discovery and development of novel antibacterial and antifungal agents[1]. The screening of plants for their biologically active principles is done on the basis of either their chemo – taxonomic investigations or ethno botanical knowledge for a particular disease[2]. Due to ever increasing prices of chemical drugs, especially in developing countries, a need to search for cheaper drug from natural sources becomes imperative[3]. In recent years more number of species have been evaluated for their antimicrobial activity.

One such vital medicinal plant (ie) *Foeniculum vulgare* Mill. (Tamil – Sompu; English – Fennel) belonging to the family Apiaceae is having lot of medicinal properties. It is distributed throughout India. The leaves are used for garnishing and leaf stalks are used in salad. The roots are used as purgative and diuretic. The fruits are sweet, acrid, bitter, emollient, expectorant, anthelmintic, carminative, digestive and stomachic. The seed extracts are used in cardiac diseases. Fennel oil is largely used as a flavouring agent in culinary preparations, confectionary and liquors[4]. The present study is aimed at screening the antibacterial properties of Foeniculum *vulgare* Mill. against the selected pathogenic bacteria.

MATERIALS AND METHODS

(i) *Preparation of Plant extracts:*

The plant material (seed) used for the study was collected from the departmental store in Tiruchirappalli district and shade dried at room temperature for 10 days. Dried seeds were powdered and 100g was taken in Soxhlet apparatus and extracted with 500ml of ethanol, methanol and aqueous (water) for seven days. The extracts were then filtered through Whatman No.1 filter paper. The filtrate was evaporated at reduced pressure to remove residual solvent and moisture. These extracts were resuspended in ethanol, methanol and aqueous to yield 100mg residue/ml solvent.

(ii) Test microbial culture & Inoculum preparation:

Seven different bacterial strains were tested for the study namely *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterobacter aerogeus*, *Salmonella typhi*, *Bacillus cereus* and *Staphylococcus aureus*. The bacterial strains were obtained from the Department of Microbiology, Institute of Basic Medicinal Science, Chennai, Tamil Nadu, India.

Nutrient agar/ Broth of Hi media Pvt. Ltd., Mumbai, India was used for culturing the bacteria and performing antibacterial testing. All the seven test bacteria were inoculated into liquid nutrient broth medium and incubated at 37 °C for 8

hrs and the suspensions were checked to provide approximately 10^5 CFU/ml.

(iii) Agar well diffusion method[5]:

15 – 20ml of nutrient agar medium was poured in the sterilized petridish and allowing it to solidify. One drop of bacterial strain was spread over the medium by L- rod wells of 6nm in diameter and about 2cm apart were punctured in the culture medium using sterile well borers. 100µl seed extract was added to the wells. Plates were incubated at 37°C for 24 hrs. Antibacterial activities were evaluated by measuring inhibition zone (cm) produced by the plant extracts.

(iv)*Disc diffusion method[6]:*

A diluted bacterial culture (0.2ml) of respective strains poured over the sterile nutrient agar. 0.2ml of each seed extract was applied per sterile filter paper disc (Whatman No.1, 6 mm in diameter) and then allowed to dry before placed on to the top layer of the agar plates. Each extract was tested in triplicate and the plates were incubated at 37°C for 24 hrs and inhibition zones were recorded.

RESULTS AND DISCUSSION

The antibacterial activity of various seed extracts were observed by measuring the diameter of the growth inhibition zone. The results are shown in Table-1. The seed extracts of *Foeniculum vulgare* Mill exhibited activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterobacter aerogeus*, *Salmonella typhi*, *Bacillus cereus* and *Staphylococcus aureus*. The inhibitory activity of the various seed extracts like ethanol, methanol and aqueous were also observed.

The results of antibacterial activity of *Foeniculum vulgare* Mill. by agar well diffusion method with the ethanolic seed extract showed the significant inhibition zone against *Enterobacter aerogeus,* moderate inhibition zone was observed against *Bacillus cereus* and followed by the other organisms. Although the seed extract with methanol showed the inhibition against *Enterobacter aerogeus*, *Bacillus cereus*, less activity was observed against *Salmonella typhi* and *Proteus vulgaris.* In the seed extract with aqueous showed high degree of inhibition zone against *Bacillus cereus, Salmonella typhi* and *Klebsiella pneumoniae* and poor inhibition was associated with *Escherichia coli* respectively.

The results of antibacterial activity of *Foeniculum vulgare* Mill. by disc diffusion method with the ethanolic seed extract showed high inhibition against *Enterobacter aerogeus,* less inhibition was associated with *Bacillus cereus* and no inhibition against *Escherichia coli, Salmonella typhi*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus vulgaris.* Whereas the methanolic seed extract showed moderate inhibition against *Enterobacter aerogeus, Salmonella typhi*, less inhibition was observed in *Bacillus cereus.* The aqueous seed extract showed high degree of inhibition against *Escherichia coli, Salmonella typhi* and moderate inhibition was observed in the same extract against *Enterobacter aerogeus* and *Klebsiella pneumoniae.*

The diameter of the inhibition zone for each sample against each micro organism were found to be either less than or greater than or equal to that of the standard antibiotic streptomycin. Among the three extracts were used, the aqueous extracts showed considerably more activity than the methanolic and ethanolic extracts. It is evident from the results of above two methods that the greater activity resides in aqueous seed extracts of *Foeniculum vulgare* Mill. against the tested bacteria followed by the ethanolic and methanolic extracts.

Similar findings and conclusions were drawn by various authors[7&8], in their experiment they found out the aqueous extract was more effective inhibitor of bacterial growth than the other extracts. Similar results were reported in *Aerva persica*[9], *Cassia occidentalis*[10] and *Achyranthes aspera*[11] also. In addition, the effectiveness of plant was not due to one main active constituent, but to the combined action of other chemical compounds involved in it[12]. Earlier reports also support the view that antibacterial activity was due to different chemical constituents including flavanoids, alkaloids, terpenoids and other compounds which are classified as active antimicrobial compounds[13]. This antimicrobial potential could be utilized in the preparation of phytomedicines in combacting diseases caused by micro organisms in plants and human beings.

Table –I

Bacterial sensitivity on Foeniculum vulgare Mill. seed extracts

S. No	Test bacteria	Extraction	Inhibition Zone (cm)	
			Agar well Mean ±SD	Disc diffusion Mean± SD
1.	*Escherichia coli*	Ethanol	2.5 ±0.16	-
		Methanol	-	-
		Aqueous	2.4 ±0.21	2.2 ±0.16
2.	*Enterobacter aerogeus*	Ethanol	2.4± 0.28	1.9 ±0.08
		Methanol	2.5± 0.37	2.1± 0.08
		Aqueous	-	2.4 ±0.32
3.	*Klebsiella pneumoniae*	Ethanol	1.6± 0.12	-
		Methanol	-	-
		Aqueous	2.1± 0.12	2.5 ±0.16
4.	*Salmonella typhi*	Ethanol	2.3 ±0.29	-
		Methanol	2.4 ±0.16	2.2 ±0.20
		Aqueous	2.1± 0.08	2.1± 0.08
5.	*Proteus vulgaris*	Ethanol	2.4± 0.21	-
		Methanol	2.4± 0.32	-
		Aqueous	-	-
6.	*Bacillus cereus*	Ethanol	2.5± 0.24	1.8 ±0.12
		Methanol	2.7 ±0.16	2.6 ±0.16
		Aqueous	2.6± 0.08	2.6 ±0.12
7.	*Staphylococcus aureus*	Ethanol	-	-
		Methanol	-	-
		Aqueous	2.2 ±0.20	-

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