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Antimicrobial activityof seed extracts of *Foeniculum vulgare* on diarrhea causing bacteria

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Summary:

The antibacterial activity of ethanolic extracts from the seeds of *Foeniculum vulgare* was tested against *Staphylococcus aureus, Escherichia coli, Salmonella enterica enterica* (Typhi), *Salmonella enterica enterica* (*Typhimurium*) and *Shigella dysenteriae* pathogenic bacteria that cause diarrhea. The Minimum Inhibitory Concentration of the seed extract against *Staphylococcus aureus* and *Escherichia coli* is 300 mg /ml while that of *Salmonella enterica enterica* (Typhi), *Salmonella enterica enterica (*Typhimurium) and *Shigella dysenteriae is* 400 mg /ml, 500mg /ml and 600mg /ml respectively.

Introduction:

Natural products, mainly the plant-derived constituents, have long been sources of drugs, and agreat part (30-40%) of the pharmaceuticals available in modern medicine is directly or indirectly derived from natural sources. Natural products are also of great interest in the process of drug discovery, due to their large diversity in nature, permitting the identification of lead molecules of greater interest for the development of new therapeutic agents, as well as biochemical and molecular tools needed to clarify complex cellular and molecular mechanisms of action involved in most physiological and pathological processes. Furthermore, agrowing world-wide interest in the use of phytopharmaceuticals as complementary or alternative medicinem either to prevent or to ameliorate many diseases, has been noted in recent years. It is believed that about 80% of world's population use plants as their primary source of medicinal agents (Mukherjee P.K., 2002; WHO, 2005).

Foeniculum vulgare is the largest genus in the family Umbelliferae and a common herb that grows in many countries especially in the Mediterranean region (Tanira *et al.*, 1996).

Foeniculum vulgare has shown a protective effect against ethanol induced gastric mucosal lesions (Birdane *et.al,* 2007). Fennel has shown anticancer (Celik and Isik, 2008); anti-inflammatory (Choi and Hwang, 2004); antioxidant (Eaudale *et.al,* 2008); antiplatelet and antithrombotic (Tognolini *et. al.*, 2006, 2007) and antispasmodic activities (Ostad *et. al.*, 2001).

Plant containing terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Kaur and Arora, 2009).This study was aimed to evaluate the antidiarrhoea property of ethanol extract of this plant in order to determine the treatment of diarrhea in folk medicine.

Materials and methods:

1- Extraction of plant materials:

The seeds, fruits and leaves of *Foeniculum vulgare* were collected from soil of Abu-alkasib and identified by Dr. Sahar Abd Al-Abaas (Department of Biology- University of Basrah).The fruits, leaves and seeds were allowed to dry at room temperature in the laboratory for a period of 2 weeks.

2-Preparation of extraction and phytochemical analysis:

Extraction from the ground dried seeds, fruits and leaves of the plant was carried out using soxhet extract. Ethanol was used as extractant.

The method of Treas and Evans (1989) were employed to test for the presence of tannins, alkaloids. The method of Harborne (1973) was used to test for the presence of steroids, saponins, glycosides and Flavonoids, Terpenoids were tested using the method of Sofowora (1993).

3- Antimicrobial assay:

After collecting of each part of the plant, Soxhlet apparatus was used for extraction. 1 liter of ethanol was used to extract 250g of *Foeniculum vulgare* at 78c˚. The extract was stored in a refrigerator at 4^{o}c until required.

The following bacteria were used in the study: *Staphylococcus aureus, Escherichia coli, Salmonella enterica enterica* (Typhimurium) ,*Salmonella enterica enterica*(Typhi) and *Shigella dysenteriae*. These organisms were collected from the microbiology laboratory of Alfaha'a Hospital –Basrah city. Cultures of these bacteria were grown in nutrient agar at 37c˚and maintained on slopes of nutrient agar. Each of the organisms was transferred into a separate test-tube containing nutrient broth to reaction them by culturing overnight at 37c˚. The different prepared extracts of the seeds of *Foeniculum vulgare* was tested for antimicrobial activity against organisms isolated from the hospital and standard strains *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (NCTC 5933) using the agar diffusion method of Thomsberry *et al.* (1983). The organisms were used to different nutrient agar plates: one organism perplate, wells made on the plates by a sterile cork borer of 6mm diameter to contan the different extracts and the plates were incubated at 37c˚for 24h. The zones of inhibition were measured at the end of the incubation period. After that the lowest concentration of the extract that inhibited the test organisms was recorded as the Minimum Inhibitory Concentration (MIC).

Results and Discussion:

The results of activity to each plant extract *Foeniculum vulgare* against standard bacteria *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (NCTC 5933), as shown in table(1).While in table (2) hows the phytochemical screening of the plant seeds . Alkaloids, Flavonoids, Glycosoides, Saponins, Steroids and Tannins were present in the ethanolic extracts of the seeds, while Terpenoids were absent in the seeds, these results are similar with researches (Kaur and Arora, 2009).

Table (1): The activity of plant extracts against standard bacteria.

Plant parts	Activity	
	Staphylococcus aureus (NCTC 6571)	*Escherichia coli* (NCTC 5933)
seeds	+++	+++
fruit	++	++
leaves	+	+

(+) Susceptibility (inhibition zone ≥ 7mm)
(++) Susceptibility (inhibition zone ≥ 14 mm)
(+++) Susceptibility (inhibition zone ≥ 21 mm)

Table (2): The result of phytochemical screening to ethanolic extract of *Foeniculum vulgare* seeds

Phytochemical compounds	Ethanolic extract
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	-

(+) presence of the compound
(-) absence of the compound

Table (3) and Figure (1) Shows the antimicrobial activity of the ethanolic extract and different concentration ranging from 100-1000 mg/ml against selected pathogenic bacteria that cause diarrhea.

Table (3): MIC of the ethanolic extract from the seeds against selected bacteria that cause diarrhea.

Microorganisms	No. of Isolates	Zones of inhibition (mm)									
		100	200	300	400	500	600	700	800	900	1000
Staphylococcus aureus	8	-	-	10	13	17	20	22	25	28	32
Escherichia coli	10	-	-	8	10	13	17	19	23	25	28
Salmonella enterica enterica(Typhi)	19	-	-	-	9	15	17	20	22	24	27
Salmonella enterica enterica (Typhimurium)	8	-	-	-	-	10	13	14	16	18	23
Shigella dysenteriae	15	-	-	-	-	-	7	10	12	15	20

The satisfactory results of MIC against *Staphylococcus aureus* and *Escherichia coli is* 300mg/ml (figure1)while *Salmonella enterica enterica*(Typhi) is 400 mg/ml (Figure 2) and 500 mg/ml,600mg/ml for *Salmonella enterica enterica*(Typhimurium) and *Shigella dysenteria* respectively(Figure3,4).

Figure (1): Antibiotic activity of seed extract against *Staphylococcus aureus Escherichia coli*

Figure (2): Antibiotic activity of seed extract against *Salmonella enterica enterica* (Typhi)

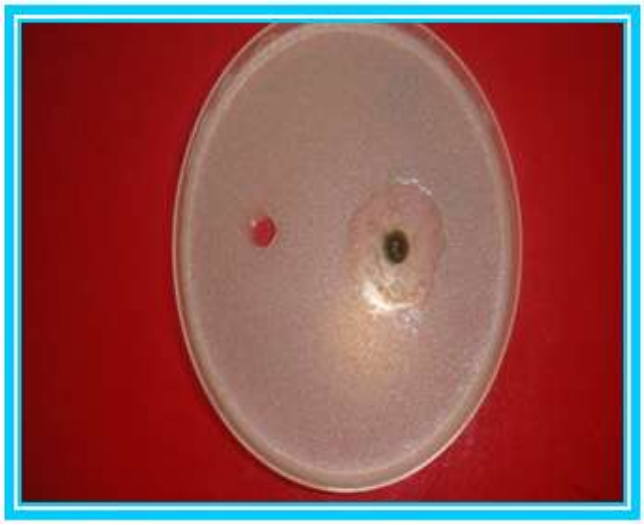

Figure (3): Antibiotic activity of seed extract against *Salmonella enterica enterica* (Typhimurium)

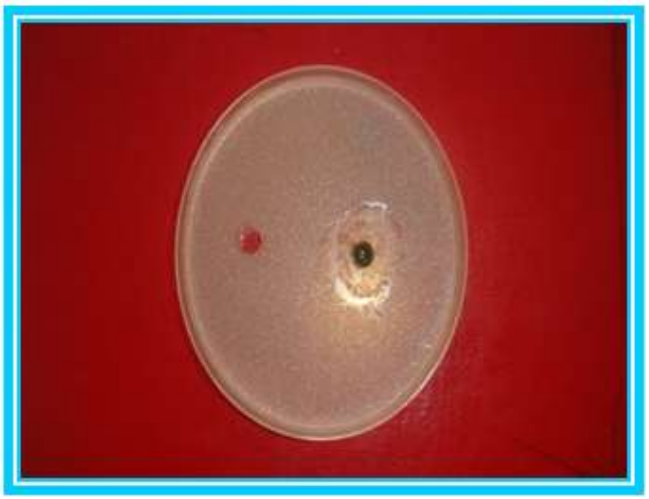

Figure (4): Antibiotic activity of seed extract against *Shigella dysenteriae*

These results are similar with Tanira *et al*.,(1996) that explained an antimicrobial agents of the ethanol extract inhibited the growth of *Staphylococcus aureus* and *Bacillus subtilis*, and they are similar with Gulfraz *et al*.,(2008) that explained antimicrobial properties of essential oil from the seed against of gram-negative bacilli. Gram-negative bacteria were sensitive to plant extracts, which may be due to their cell wall composition (Cantore *et. al.*,2007)

Recently, there is agrowing interest in finding natural antimicrobial compounds as an alternative to synthetic substances and many studies have reported that plant contain awide variety of compounds with beneficial health effects. We conclude from this research, that the plant extract has displayed antimicrobial activity and therefore justifies its botanical uses for the treatment of coughs, colic, hemorrhoids, ulcer. For Future study on this plant, the active ingredients of the plant should be investidated.

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الفعالية المضادة للجراثيم لمستخلص بذور *Foeniculum vulgare* تجاه الجراثيم المسببة للاسهال

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الخلاصة:

أختبرت فعالية المستخلص الايثانولي لبذور نبات *Foeniculum vulgare* تجاه الجراثيم المرضية *Staphylococcus aureus*، *Salmonella enterica enterica* (Typhimurium), *Salmonella enterica enterica*(Typhi)، *Escherichia coli* و *Shigella dysenteria* المسببة للاسهال. وجد ان التركيز المثبط الادنى لمستخلص البذور تجاه *Staphylococcuc aureus* و *Escherichia coli* كانت 300 ملغم/مليلتر بينما بلغت تجاه (Typhi)*Salmonella enterica enterica* , *Salmonella enterica* (Typhimurium) و *Shigella dysenteria* (400 ملغم/مليلتر, 500 ملغم/مليلتر ،600 ملغم/مليلتر)على التوالي .