Antibacterial Effect of Dill Seed Oil Anethum graveolens Anethum graveolens
التأثير المضاد البكتيري لزيت بذور الشبنت

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الخلاصة

خلفية البحث: دراسة الفعالية المثبطة لزيت بذور نبات الشبنت Anethum graveolens على نمو بعض أنواع البكتريا المرضية.

الهدف: اختبار الفعالية التثبيطية لزيت بذور نبات الشبنت في بعض انواع البكتريا المرضية المسببة للالتهابات المختلفة.

المنهجية: نفذت تجربة لدراسة النشاط المضاد البكتيري لزيت بذور الشبنت في نمو ست عزلات من البكتريا المرضية: Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis المعزولة من مرضى يعانون التهابات مختلفة من كلا الجنسين تتراوح اعمارهم من 15–50 سنة، بطريقة الحفر، وتم اختيار العينات من المختبرات التعليمية في مستشفى بغداد التعليمي، وأجريت الدراسة في شهر تشرين الثاني من العام 2014 إلى شباط 2015. وتم تحليل البيانات بواسطة مقياس ليكرت، بالإضافة الى فحص الحد الأدنى من التركيز المثبط (MIC).

النتائج: ان زيت بذور الشبنت أختــزل معنويـــا نمــو جميع أنواع بكتيريا الاختبار E. faecalis (15 -25 ملم)، P. mirabilis (20-25ملم) ، P. aeruginosa (17 -27 ملم)، K. pneumonia (15 -35 ملم)، Escherichia coli (20 -52 ملم) وشوهدت أوسع منطقة تثبيط في بكتريـــاS. aurous (70- 72 ملم).

الاستنتاج: ان زيت بذور الشبنت له تأثير مثبط واضح في نمو البكتريا المرضية الموجبة والسالبة لصبغة كرام.

التوصيات: إجراء المزيد من الدراسات على زيت بذور الشبنت لتطوير عقار جديد من نبات الشبنت لعلاج الأمراض التي تصيب الإنسان نتيجة لفعاليته في تثبيط نمو البكتيريا وتقليل استعمال المضادات الحيوية بسبب مشكلة مقاومة البكتيريا والآثار الجانبية، وأنه من الضروري تحديد سمية المكونات النشطة والخصائص الدوائية.

ABSTACT

Background: study the inhibiter activity of Dill seed oil A. graveolens L. on growth of some pathogenic bacteria.

Objective: assay the inhibition effectiveness of Dill seed oil on some pathogenic bacteria which causes different infections.

Material and Method: an experiment was run to study the antibacterial activities of Dill seed oil on six isolates of bacteria: E. coli, K. pneumonia, P. mirabilis, P. aeruginosa, S. aureus, , and E. faecalis which isolated from patients suffer from different infections of both genders their age ranged (15-50) year, used agar well diffusion method. The isolates selected from Baghdad Teaching Hospital-Teaching Laboratories from December 2014 to the February 2015. Data was analyzed by using the Lycart scale analysis, and assays the Minimum Inhibitory Concentration (MIC).

Results: Dill seed oil significantly reduced the growth of test bacteria: E. faecalis (15-25mm), P. mirabilis (20-25mm), P. aeruginosa (17-27mm), K. pneumonia (15-35mm), E. coli (20-52mm), and the widest inhibition zone diameters was showed on S. aureus (70-72mm).

Conclusion: Dill seed oil has clear inhibition effect on the growth of Gram-positive and Gram-negative pathogenic bacteria.

Recommendation: done further studies on dill seed oil for development of novel drugs from A. graveolens to treat human diseases as a result of its inhibition effectiveness on growth of bacteria and decreasing the use of antibiotics because the problem of resistant of bacteria and side effects, and it is necessary to determine the toxicity of the active constituents and pharmacological -kinetic properties.

Key wards: Dill seed oil, Anethum graveolens, Pathogenic bacteria, MIC.

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INTRODUCTION

Dill Anethum graveolens, is one of the family Umbelliferae plants, an annual herb and it's originates from West Asia and Mediterranean. It is used as a source of essential oil and as a vegetable. Its seeds are used in tea, breads, soups. Hence, there has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils **(1).**

Dill has many medicinal uses: antibacterial, antispasmodic, antitumor, calming, digestive, disinfectant, expectorant, laxitive (mild), diuretic, carminative, stimulant and has hypolipidemic activity it could be used as a cardioprotective agent. The main constituents of dill oil which is pale yellow in color, darkens on keeping, with the odor of the fruit and a hot, acrid taste are a mixture of a paraffin hydrocarbon and 40 to 60% of d-carvone (23.1%) with d-limonene (45%). It also consists of α-phellandrene, eugenol, anethole, flavonoids, coumarins, triterpenes, phenolic acids and umbelliferones. The chemical composition of dill essential oil and the amount varies depending on the plant parts and the developing stage of the plant at harvest time **(2, 3).**

There are largest number of bacterial cells in the human flora which being in the gut flora, and a large number on the skin **(4).** The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and some are beneficial, but there are several species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy and bubonic plague. In developed countries, antibiotics are used to treat bacterial infections, making antibiotic resistance a growing trouble furthermore the side effaced of it **(5).**

The prevalence of drug resistant pathogens is one of the important menace to successful treatment of microbial diseases. The essential oils and other extracts of plants have raised benefit as sources of natural products. They have been examined for their potential uses as alternative solution for the treatment of many infectious diseases **(6).**

The problem of side effects and expansion of bacterial resistance to synthetic antimicrobial factors associated with use alternative agent or complementary like essential oils. Dill is a plant with wide range of chemical constituents which exerted many pharmacological effects to treat infections caused by multi-drug resistant strains of microorganisms from community as well as hospital settings. There is a great promise for development of novel drugs from A. graveolens to treat human diseases as a result of its effectiveness **(7).**

In Iraq, reported that the most prevailing pathogenic microorganisms is the antimicrobial resistance, therefore the present study relates to test Dill seed oil as antibacterial activity against Gram-positive bacteria, S. aureus and E. faecalis and Gram-negative bacteria, K. pneumonia, E. coli, P. aeruginosa, P. mirabilis, which isolated from various human infections, and WHO has developed, a draft global action plan to combat

antimicrobial resistance which has been submitted to the sixty-eighth World Health Assembly, taking place in May 2015.

MATERIALS AND METHODS

An experiment was run to study the antibacterial activities of Dill seed oil on six isolates of bacteria: E. coli, K. pneumonia, P. mirabilis, P. aeruginosa, S. aureus, and E. faecalis which isolated from patients suffer from different infections of both genders their age ranged (15-50) year, from December 2014 to the February 2015.

Dill Oil Extraction

Dill seeds was purchased from Al-Shorja market, removed the bad seeds and washed in colander with water then dried and powdered in mortar and prepared by steam distillation. The oil was further distilled by rotary evaporator, then transferred into sterile vials and stored in refrigerator until used [6,7].

Figure (1): The Seeds of Dill plant Anethum graveolens

Bacteria Diagnosis

Identification of bacteria isolates based on cultural characteristics, microscopically examination by observing details developing colonies in size, height, shape and color, growth on selective media and standard biochemical tests [8].

Bacterial culture preparation

Pure cultures of the study bacteria (E. coli, K. pneumonia, P. mirabilis, P.aeruginosa, S. aureus and E. faecalis were obtained from Baghdad Teaching Hospital/Teaching laboratories, the cultures of bacteria were maintained in appropriate agar slants at 4°C and sub-cultured from nutrient broth for 24 hour prior to testing.

Antibacterial bioassay

The antibacterial activity of Dill seed oil was done using agar well diffusion method. Muller Hinton agar plates were inoculated with 0.1 ml of each bacterial organism (1×10^8CFU/ml) and spreaded well with sterile swabs. Wells of 6 mm size were made into the agar by cork porer, set plates containing the bacterial culture. Subsequently, wells were filled with 100 µl of dill oil and allowed to diffuse at room temperature for about 2 h. The plates were incubated at 37ºC for 24 h. The control well containing the same volume of

paraffin oil. After incubation, the zone of inhibition was measured and expressed in millimeter [9]. All tests were performed in triplicate and the antibacterial activity was expressed by the Lycart scale analysis.

Minimum Inhibitory Concentration (MIC)

The Minimum inhibitory concentration (MIC) used to test the essential oils of Dill seed for the most susceptible bacteria (E. coli and S. aureus), determined by application the agar dilution method to assay the lowest concentration of essential oil for microbiology growth inhibition of the tested microorganism [10]. Prepared by put different concentrations of Dill seed oil in each petri plate containing nutrient agar: 0.5, 1, 1.5, 2, 2.5 3 3.5 4 4.5 and 5 µl/ml which equal 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 ppm.

RESULTS

Table (1): **Antibacterial activity of Dill seed oil expressed by mean of score.**

No.	Test Bacteria	Inhibition zone (mm) (mean)*	Control (paraffin oil)	Zone of inhibition diameter (mm)			M.S.	S.
				1	2	3		
1.	Enterococcus faecalis	21	0.0	15	23	25	0.75	N.S.
2.	Pseudomonas aeruginosa	23	0.0	17	25	27	1.48	N.S.
3.	Proteus mirabilis	24	0.0	23	24	25	1.46	N.S.
4.	Klebsiella pneumonia	25	0.0	15	25	35	1.7	S.
5.	Escherichia coli	38	0.0	27	35	52	2.53	H.S.
6.	Staphylococcus aurous	72	0.0	71	72	73	4.44	H.S
	Total			168	204	237	2.06	S.
	X^2 obs. = 12.271	df = 5		X^2 crit. =11.070			P < 0.050	

* mean of 3 plates N.S.=No Significant S.= Significant H.S.= High Significant M.S. = Mean of score

Table (1) shows that the Dill seeds oil had an antimicrobial activity against tested bacteria: E. faecalis (21mm), P. aeruginosa (23mm), P. mirabilis (24mm), K. pneumonia (25mm), E. coli (38mm), and the widest inhibition zone was showed on S. aureus (72 mm), respectively. The control plate did not exhibit inhibition zone. The current study revealed that the Dill oil shows high significant against S. aurous, the maximum inhibition zone was found to be (72mm) in diameter followed by E. coli (38mm), to significant antibacterial activity against K. pneumonia (25mm), while no significant toward E. faecalis (21mm), P. aeruginosa (23mm) and P. mirabilis (24mm).

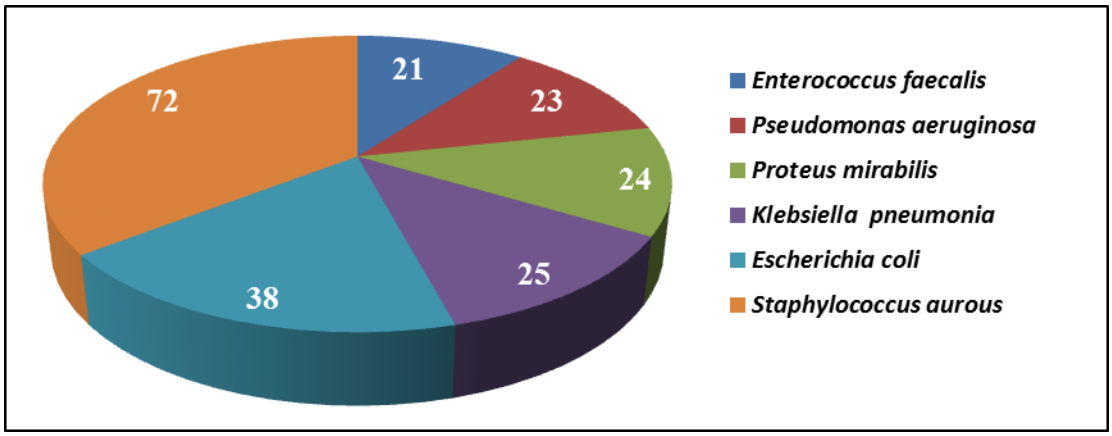

Figure (2): Shows the inhibition zone of the test Bacteria in diameter (mm).

The biological activity test against microorganisms indicated that the Dill seed oil toxic to Escherichia coli (38mm) and Staphylococcus aurous (72mm).

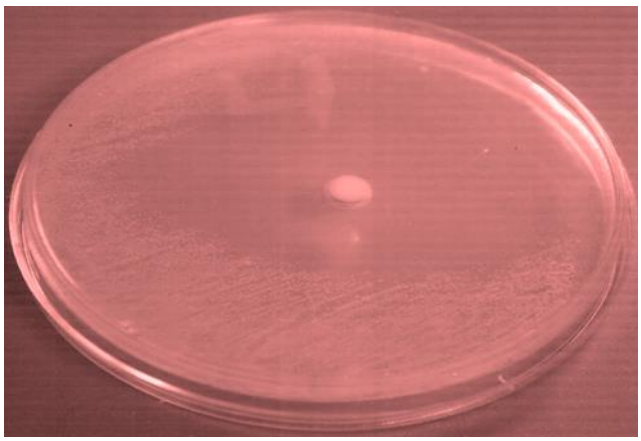

Figure (3.a): Effect of Dill oil on Staphylococcus aureus (Inhibition zone 72 mm)

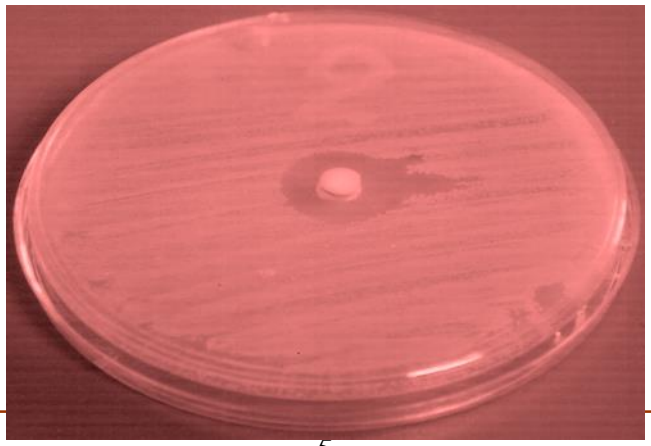

Figure (3.b): Effect of Dill oil on Escherichia coli (Inhibition zone 38 mm)

Table (2): Minimum Inhibitory Concentration (MIC) Value of Dill seeds oil against the most susceptible tested bacteria.

Microorganisms	Concentration of Dill seed oil (µl/ml)									
	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Escherichia coli	+ +	+ +	+	-*	-	-	-	-	-	-
Staphylococcus aurous	+ +	+	-*	-	-	-	-	-	-	-

+ + bacteria growth + low bacteria growth *no bacteria growth

Table (2) shows maximum efficacy of Dill seeds oil with Minimum inhibitory concentration (MIC) values starting from 0.5 to 5 µl/ml. Against E. coli the MIC value is 2 µl/ml, while S. aurous the MIC value is 1.5 µl/ml.

Generally, E. coli and S. aureus are the most sensitive bacteria, while E. faecalis, P. aeruginosa, P. mirabilis and K. pneumonia showed low level of susceptible.

DISCUSSION

Seed of A. graveolens contain 4% essential oil include the major compounds: limonene (33%), carvone (30 - 60%), α-phellandrene (20.61%), including dihydrocarvone, diterpene, pinene, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, myristin, apiol and dillapiol, furanocoumarin, 5-(4”-hydroxy-3”methyl-2”-butenyloxy) -6,7-furocoumarin, oxypeucedanin, oxypeucedanin hydrate and falcarindiol [(11)].

The efficacy of the oils would be prospective to relate to the particular composition of plant volatile oils, the structural arranging of constituent components of volatile oils and their functional groups and potential synergistic interactions between components [(12,13)].

Essential oils usage could be a source of pharmaceutical materials required for the production antimicrobial agents structure and a new therapeutic, such as thymol, carvacrol, terpenoids, eugenol, and were highly active against the microorganisms. Furthermore, many researchers investigated the effect of volatile oils and mode of action of essential oils [(6)].

The previously studies on essential oil of A. graveolens revealed its antimicrobial potential [(14,15)], that’s may be the terpenoids phenol and flavonoid exhibit effectiveness against the pathogens potentially acting as solvents or dehydrating agents or protein denaturing agents, Terpenoids and phenol are known to possess vigorous antimicrobial activity. The antimicrobial activity of essential oils is assigned to a number of small terpenoids [(12)].

CONCLUSION

Dill seed oil has clear inhibition effect on growth of positive-Gram and negative -Gram bacteria, it’s a plant with wide range of chemical constituents which exerted many

pharmacological effects to treat infections caused by multi-drug resistant strains of microorganisms from community.

RECOMMENDATIONS

1. Further studies should be done on dill seed oil for development of novel drugs from Anethum graveolens to treat human diseases as a result of its inhibition effectiveness on growth of bacteria and decreasing the use of antibiotics because the problem of resistant of bacteria and side effects.
2. It is necessary to determine the toxicity of the active constituents and pharmacological -kinetic properties.

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