

## In vitro effect of alcoholic extract of *Pimpinella anisum* against some bacterial growth

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### Abstract

Alcoholic extract of *Pimpinella anisum* fruit have been prepared at different concentration (10, 25, 50, 100, 200) mg/ ml.

The extract of plant tested in vitro against (7) bacterial given by disc diffusion method *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The result have been shown that the effect of extract at concentration of 200 mg/ml gave most inhibition zone on *Pseudomonas aeruginosa* (18) mm, and less than in case of *Salmonella typhimurium* (17) mm, *Staphylococcus aureus* (14) mm, *Escherichia coli* (13) mm, *Klebsiella pneumonia* (9) mm.

At concentration (100) mg/ml, the growth inhibition zone was (12) mm in case of *Pseudomonas aeruginosa*, *Staphylococcus aureus* (10) mm, and *Escherichia coli* (10)mm. At concentration 50 mg/ml inhibition zone was 9 mm for *Staphylococcus aureus*, *Escherichia coli* 10 mm, while for concentration of 25 and 10 mg/ml the inhibition zone was (8), (10) mm for *Staphylococcus aureus* growth respectively.

### تأثير المستخلص الكحولي لنبات الينسون ضد نمو بعض البكتريا في الزجاج

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### الخلاصة

حضر المستخلص الكحولي لنبات الينسون بتركيز مختلفة (50، 25، 10، 100، 200) ملغم/مل. تم اختبار المستخلص الكحولي لنبات الينسون لتثبيط النمو البكتيري لسبعة أجناس بكتيرية *Escherichia coli* ، *Staphylococcus aureus* ، *Proteus vulgaris* ، *Listeria monocytogenes* ، *Salmonella typhimurium* ، *Pseudomonas aeruginosa* و *Klebsiella pneumonia* . أظهرت النتائج تأثير المستخلص الكحولي بتركيز 200 مايكرو غرام/مل إذ بلغ قطر التثبيط (18) ملم لجرثومة الزوائف الزنجارية، (17) ملم لجرثومة السالمونيلا تيفيموريوم، (14) ملم لجرثومة المكورات الذهبية العنقودية، (13) ملم لجرثومة الايشيريشيا القولونية و (9) ملم لجرثومة الكلبسيلا نيمونيا. أما بتركيز 50 مايكرو غرام/ مل فقد بلغ قطر التثبيط (9) ملم للمكورات الذهبية العنقودية و (10) ملم للأيشيريشيا القولونية. أما بتركيز 25 و 10 مايكرو غرام/ مل فقد بلغ قطر التثبيط لجرثومة المكورات الذهبية العنقودية (8) و (10) ملم على التوالي.

### Introduction

Plants are a large source of new bioactive constituent, with therapeutic potential. Only small percentage of living plants on earth have been phytochemically investigated.

Plants are thus enormous reservoir of pharmaceutically valuable molecules to be discovered (1,2).

As an aromatic plant, anise (*Pimpinella anisum* L.) is an annual herb indigenous to Iran, India, Turkey and many other warm region in the world and have been used traditionally in therapy of some disease world wide for long time; *Pimpinella anisum*

has been used as a stimulating effect of digestion and antiparasite, antifungal (3) and for treat catarrh of respiratory tract and as mild expectorant ,and it was reported that extract from anise fruit have therapeutic effect on several condition such as a gynecological and neurological disorder (4,5) and can be used as antipyretic (6); additionally the plant and especially it fruit essential oil have been used for treatment of some disease including seizures and epilepsy (7, 8). The present investigation was performed to evaluate antibacterial activity of anis, Flax alcoholic extract against some pathogenic bacteria.

### Material and Methods

- 1. Plants:** The fruit of *Pimpinella anisum* brought from local marked and verified by Iraq National Herb, Agricultural Ministry.
- 2. Extraction:** The plants were first dried and ground to powder, then (100) of each plants soaked in 500ml of 70% ethanol, then separated using separator funnel, subsequently filtered through whatman filter paper No.1 and filtrate dried, the dried extracted was weight for prepare the stock solution by dissolved (4g) of each extract powder in (20ml) of distal water to prepare concentration 200 mg/ml from which another concentrations (100, 50, 25, 10) mg/ml were prepared.
- 3. Microorganism:** The test organisms were obtained from unit of Zoonotic disease, which included *Escherichia coli*, *Staphylococcus aureus*, *Proteus sp.*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsella pneumonia*.

The organism were identified by culture and biochemical characteristic shown in Table (1).

**Table(1) biochemical test for identification of isolated bacteria**

Bacterial strain	Biochemical test						
	Gram stain	catalase	oxidase	urease	coagulase	TsI	IMVic
<i>Staphylococcus aureus</i>	+ve	+ve	_ve	+ve	+ve	Y/Y	+ -
<i>Escherichia coli</i>	_ve	+ve	_ve	_ve	_ve	Y/Y	+ -
<i>Proteus sp</i>	_ve	+ve	_ve	+ve	_ve	P/Y H2S+	+ -
<i>Listeria monocytogenes</i>	+ve	+ve	_ve	_ve	_ve	Y/Y	+ -
<i>Salmonella typhimurium</i>	_ve	+ve	_ve	_ve	_ve	P/Y H2S+	+ -
<i>Pseudomonas aeruginosa</i>	_ve	+ve	+ve	+ve	_ve	P/P	- -
<i>Klebsiella pneumonia</i>	_ve	+ve	_ve	+ve	_ve	Y/Y	- +

The sensitivity of isolated pathogen for different antibiotics were shown in Table(2).

**Table (2) resistant or susceptibility of bacterial strain to antibiotic**

Antibiotic	Inhibition Zone Diameter (mm)
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	<i>Pseudomonas aeruginosa</i>	<i>Proteus sp</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Listeria monocytogenes</i>	<i>Klebsiella</i>	<i>Salmonella typhimurium</i>
Ampicillin 10Mcg	5 (R)	9	19	10	23	5	5
Vancomycin 30 Mcg	5 (R)	5 (R)	18	5	28	5	5
Erythromycin Mcg 15	5 (R)	5 (R)	29	14	36	9	8
Imipene 10Mcg	25	20	39	24	38	32	5
Cefotaxime 30 Mcg	8	20	9	18	10	29	26
Streptomycin 10Mcg	20	5 (R)	11	19	30	20	9
Clindamycin2 Mcg	7	5 (R)	16	5	20	5	5
Rifampin 5Mcg	5 (R)	5 (R)	35	8	32	9	5

R=Resistant.

**4. Culture media:** The culture media were used Muller Hinton agar and Nutrient agar for bacterial growth.

The bacterial cultured inoculated into Brain heart infusion broth and incubated at 37C° for 24 hours. The inoculums was standardized according to McFarland turbidity standard. The turbidity was compare with McFarland 0.5 standard which provide turbidity comparable to bacterial suspension containing  $1.5 \times 10^8$  CFU/ml (9).

**5. The disc diffusion technique:** This test was performed using the standard procedure as described by (10) the inoculums suspension of each bacterial strain was swabbed on the entire surface Muller Hinton agar.

Sterile 7 mm filter paper discs were aseptically placed on Muller Hinton agar surface and crud ethanolic extract were immediately added to disc in concentration (10, 25, 50, 100, 200) mg/ml. Then left at ambient temperature for 15 min to allow excess pre diffusion of extract prior to incubation at 37C° for 24 hours. Diameter of growth Inhibition Zone for different bacteria were measured for each one in duplicate.

### Result and Discussion

The result of study of effect of ethanolic extract of *Pimpinella anisum* fruit on some pathogenic species of bacterial growth were tested in (Table3) which showed that the concentration of 200 mg/ml cause strongest effect by gave high inhibition zone on the *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *E.coli*, *Klebsiella* (18,17,14,13,9) mm respectively while at concentration 100 mg/ml the inhibition zone was (12) mm in case *Pseudomonas aeruginosa*, (10) mm in *Staphylococcus aureus* and (10) mm in case *E.coli*.

**Table (3) effect alcoholic extract of *Pimpinella anisum* on bacteria**

Concentration/ mm	Mean of inhibition zone (mm)						
	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Proteus sp.</i>	<i>Listeria</i>	<i>Salmonella typhimurium</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>
10	10	-	-	-	-	-	-
25	8	-	-	-	-	-	-
50	9	10	-	-	-	-	-
100	10	12	-	-	-	12	8
200	14	13	-	-	17	18	9

At concentration of 50 mg/ ml inhibition zone was (9) mm at *Staphylococcus aureus*, *E.coli* was (10) mm, and other bacteria give negative result.

The result of the present study support the evidence that mentioned by other researchers like (11) who found that methanolic extract of *Pimpinella anisum* showed

antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *E.coli* and *Klebsiella pneumonia* (8,11,11,9) mm in diameter respectively where as acetone and petrolenum other extracted was not observed to inhibit the growth of any of test bacteria under study; while (12) who reported that the antibacterial activity of anis was investigated on gram positive bacteria *Staphylococcus aureus*, *Streptococcus epidermidis*, *Streptococcus pneumonia*, *Micrococcus luteus* and gram negative bacteria *Proteus mirabilis*, *Citrobacter koseri*, *Enterobacter aerogenes* the extract showed no bactericidal effect on *Pseudomonas aeruginosa* and *E.coli* and the result of effect of ethanolic extract of anise fruit belong to it contains which record by (13) who which included trans-anethol, methylchavicol estragole), eugenol, psedoisoeugenol, anisaldehyde, coumarins (umbelliferon, scopoletin) caffeic, acid derivatives (chlorogenic acid) flavonoid, fatty oil, proteins, minerals, polyenes and polyacetylenes as its major compounds.

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