تطبيقات المكونات الكيميائية المستخلصة من نبات اليانسون لمضادات البكتريا والفطريات.

Application of the chemical constituents Extracted from Pimpinella anisum plant as bacterial activity and fungal activity

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الملخّـــص :

في هـــــــــذا العمل تم تجفيف أجزاء نبات *اليانسون* *Pimpinella anisum* 200.0 جم) في الظل عند درجة حرارة الغرفة (22) درجة مئوية وطحنها جيدًا، كذلك تم عمل استخلاص كيميائي بكميات متساوية من / Pet.Ether / $CHCl_3$ MeOH (3 × 1 لتر) في درجة حرارة الغرفة لمدة أسبوعين. بعد ذلك تم عمل تبخير للمذيبات المذكورة تحت ضغط منخفض بواسطة المبخر الدوار حيث تم الحصول على 87. 55 جم من المستخلص الخام لنبات *الينسون* ، كمية 66.24 جم من مستخلص Pet.Ether / $CHCl_3$ / MeOH لنبات *الينسون المذابة* جيدًا في مذيب مائي (H_2O) والتي تم الحصول عليها بعد استخلاصها جيدًا ثلاثة مرات عن طريق الفصل بالقمع باستخدام ثلاثة مذيبات عضوية مختلفة (Pet. Ether و Chloroform و Ethyl Acetate) للحصول على ثلاثة كسور من النبات : (أ – ب – ج)

(أ) جزء البتروليوم ايثير 19.57جم (ب) جزء الكلوروفورم 32.13جم

(ج) جزء خلات الإيثيل 14.54جم

تم عمل وصف لهذه المستخلصات باستخدام تقنية GC/MS والتي من خلالها تم التعرف علي (39) مركب كما في **الجـــــدول (1).**

تمت دراســــة : الأنشـــطة المضادة للميكروبات للمستخلصات من نبات الينسون. تم إذابة كل مستخلص بعد تبخيره وتجفيفه في ثنائي ميثيل سلفوكسيد

(DMSO) لتكوين تركيزات 1 و 10 و 25 و 50 مجم / مل، ثم تم فحصـها بحثًا عن وجود أنشـطة مضـادة للميكروبات باسـتخدام خمسـة بكتيريا ممرضـة منها الايجابية لصبغة جرام مثل *Staphylococcus aureus* و *B. cereus* والسالبة لصبغة جرام مثـل *Escherichia coli*، *Pseudomonas aeruginosae* و *Klebsiella pneumonia*. وأيضـا تم فحص جميع المسـتخلصـات كمضـادات للفطريات باسـتخدام أنـواع مـخـتلفـة من الـفطريـات المســببـة للأمراض: *Candida albicans* ، *Penicillium notatum*، *Alternaria alternata*، *Aspergillus niger* و *Fusarium oxysporium- pisi*.

لوحظ أن الأنشـطـة المضـادة للبكتيريا والفطريات لجميع المركبات تزداد خطيًا مع زيادة تركيز المسـتخلصـات . وجد أن الكلوروفورم يثبط نمو جميع الكائنات الحية الدقيقة وأظهر التأثير مدى واسـع ضـد كل من البكتيريا موجبة الجرام (*S. aureus* ؛ 12 مم و *B. cereus* ؛ 15 مم) و البكتيريا سـالبة الجرام (*P. aeruginosa* ؛ 18 مم). ومن ناحية أخرى كان مستخلص الأثير البترولي ومستخلص ETOAC وجميع مستخلصات *S. aromaticum* خالية تمامًا من أي نشاط ضد بكتريا الالتهاب الرئوي *K. pneumonia*، من نـاحيـة أخرى كـان أعلى نشـاط مضـاد للفطريـات لجميع المستخلصات هو 25 مم و 22 مم ضد *P. notatum* و *C. albicans* على التوالي. أظهر التحليل الإحصـائي أن تأثير نوع المسـتخلص كان أقوى (بنسـبة F أعلى) من التركيز لجميع الأنواع البكتيرية والفطرية. بينما في *S. aromaticum* كـان تـأثير التركيز أقوى (بنسبة F أعلى) من نوع المستخلص لجميع الأنواع البكتيرية والفطرية.

تم فحص القدرة الكلية المضادة للأكسدة للمستخلصات المختلفة بتركيزات مختلفة من المستخلصات ، لوحظ أنه مع زيادة تركيز أي من هذه المستخلصات، زاد نشاط مضادات الأكسـدة.

Abstract.

The present work aims to study focused on the isolation of secondary metabolites from, *Pimpinella plant* which exhibits antimicrobial or cytotoxic activities. Various chromatographic techniques such as TLC and CC were used to extract and purify the interesting compounds. Elucidation of the chemical structures of the compounds was carried out by interpretation of their spectroscopic data (e.g IR, MS, 1D and 2D NMR). The required stereochemistry of the compounds was assigned. The biological activities

such as antibacterial, antifungal and cytotoxic of the isolated compounds were also explained.

Introduction

Anise seed is widely used to flavour pastries; it is the characteristic ingredient of a German bread called *anisbrod*. In the Mediterranean region and in Asia, aniseed is commonly used in meat and vegetable dishes. It makes a soothing herbal tea and has been used medicinally from prehistoric times. The essential oil is used to flavour absinthe, anisette, and Pernod liqueurs.

Descreption

Pimpinella anisum also called aniseed or rarely anix, is a flowering plant in the family Apiaceous native to the eastern Mediterranean region and Southwest Asia. The *Pimpinella anisum* has common names in different countries such as Anise vert (France); Anise seed (Japan); Anise and Star anise (the USA); Annabella (Italy); Anisa, Badian, Kuppi, Muhuri, Saunf and Sop (Iran and India); Boucage anise, Petit anise (North Africa), and anise (England). [1]

Anise is an herbaceous annual plant growing to 90 cm (3 ft) or more. The leaves at the base of the plant are simple, 10–50 mm ($\frac{3}{8}$–2 in) long and shallowly lobed, while leaves higher on the stems are feathery pinnate, divided into numerous small leaflets. The flowers are either white or yellow, approximately 3 mm ($\frac{1}{8}$ in) in diameter, produced in dense umbels. The fruit is an oblong dry schizocarp, 3–6 mm ($\frac{1}{8}$–$\frac{1}{4}$ in) long, usually called "aniseed".

The flavor and aroma of its seeds have similarities with some other spices, such as star anise, fennel, and licorice. It is widely cultivated and used to flavor food, candy, and alcoholic drinks, especially around the Mediterranean. Anise is a food plant for the larvae of some of Lepidoptera species (butterflies and moths), including the lime-speck pug and warm wood pug.

Experiment

Processing of Pimpinella

The parts of plant Pimpinella, (200.0 g) was dried in the shade at room temperature 22 ℃ and grinded well, then extracted with equal volumes of Pet.Ether/$CHCl_3$/MeOH (3 × 1 L) at room temperature for 2 weeks.

Evaporation of the solvents under reduced pressure by rotary evaporator provided (87. 55 g) oily residue respectively for pimpinella.

Applying Liquid-Liquid Extraction technique for the plant *Pimpinella.*

65.66 g of the crude Pet.Ether/CHCl3/MeOH extract of *Pimpinella* dissolved well in aqueous solvent (H2O), then extracted well three times by separating funnel using three different organic solvents (Pet. Ether, Chloroform and Ethyl Acetate) to afford three fractions (**A**-**C**).

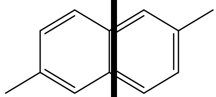
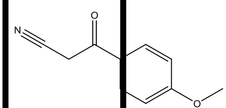
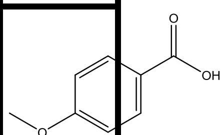
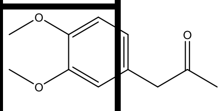
Pet.Ether fraction (**A**): 19.57 g

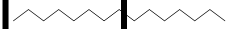
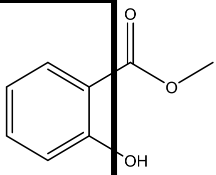
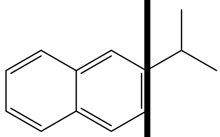
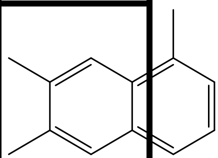
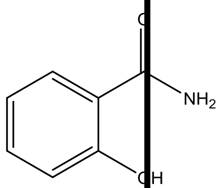
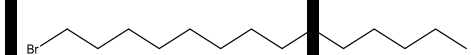
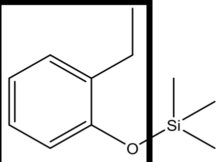
Chloroform fraction (**B**): 32.13 g

Ethyl Acetate fraction (**C**): 14.54

The biological activity of these fractions has been studied, and the major compounds of the crude Pet.Ether/CHCl3/MeOH extract of *Pimpinella* have been identified using GC/MS

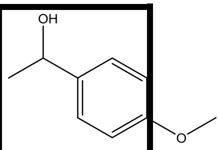
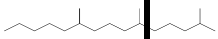
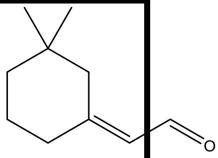
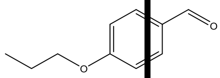
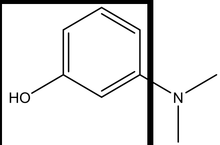
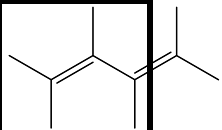
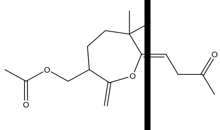
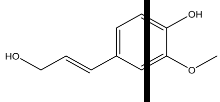
Table1 Chemical constituents identified by GC/MS technique from Pet.Ether/$CHCl_3$/MeOH extract of *Pimpinella* whole plant material

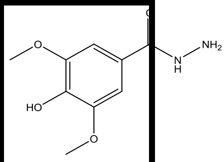
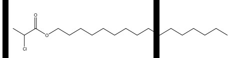
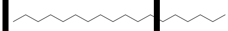
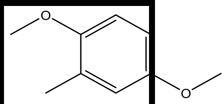
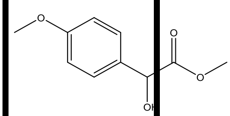
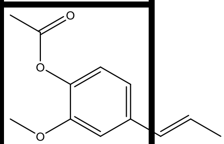
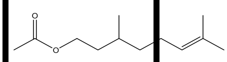
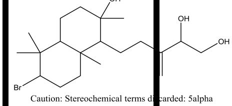
No	Compound Name	Rt	Mol. Wt	Area %	MS-Data	CSBC
1	Naphthalene, 2,6-dimethyl-	5.335	156	0.33	156 µ+ (100%),141(88),128(21),125 (25),102(3),89(3),77(13), 63(13)51(7)39(3),27(2).	
2	4-Methoxybenzoylacetonitrile	5.665	175	1.85	175(13),137 µ+ (100%),121(13),107(21),92 (27),77 (13),64(21),50(6),28(4), 15(4).	
3	Benzoic acid, 4-methoxy	5.756	152	0.89	152(98),135 µ+ (100%),107(17)92(6),77(17),63(6), 50(3),39(3).	
4	3,4-Dimethoxyphenylacetone	6.003	212	4.48	212(2),141(2),113(5),85(39), 57 µ+ (100%),41(75),26(16).	

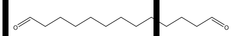
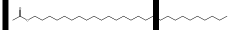
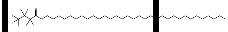
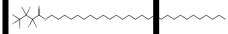
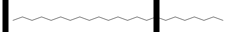
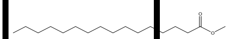
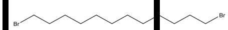
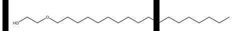
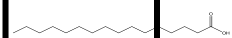
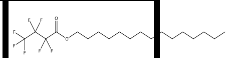
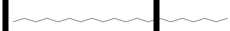
5	Pentadecane	6.249	152	0.17	152(50),120 μ+ (100%),92(72),65(25),53(7),39(18).	
6	Methyl salicylate	6.299	152.1	0.92	152(50),120 μ+ (100%),92(72),65(25),53(7),39(18).	
7	Naphthalene, 2-(1-methylethyl)	6.323	170	0.38	170(28),155 μ+ (100%),141 (4),128(16),115(10),102(2), 89(1),76(10),63(4),51(3),39(2), 27(2).	
8	Naphthalene, 1,6,7-trimethyl	6.529	170	0.85	170(28),155 μ+ (100%),141 (4),128(16),115(10),102(2), 89(1),76(10),63(4),51(3),39 2),27(6),15(4).	
9	Salicylamide	6.683	137	0.13	137(73),120 μ+ (100%),92(87),80(4),65(28),53 (12),39 (28),27(2)	
10	Tetradecane, 1-bromo-	6.746	277	0.27	163(2),135(60)110(5),85(34), 57 μ+ (100%),29)27).	
11	2-Ethylphenol, tri methyl silyl ether	6.907	194	3.29	194(73),179 μ+ (100%),163(63),149(15),135(1 6),121(2), 105(10),91(12),73(33),59(9),45 (1), 27(10).	

Antibacterial activity screening

The bacterial infection causes a high rate of mortality in the human population and aquaculture organisms [2]. In our results, varying amounts of

12	4-Methoxyphenyl methyl carbinol	7.645	152	0.60	152(29),137 µ+ (100%),122(2),109 (60),94(41),77(45),65(19),43(48),27(6), 15(13).	
13	Pentadecane, 2,6,10-trimethyl	7.937	254	0.47	183(2),141(3),113(9),85(28),57 µ+ (100%),29(19).	
14	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (Z)-	8.040	152	0.10	152(28),137 µ+ (100%),123(4),10995(24),81(44),69(44),55(23),41(56), 27(12).	
15	4-Propoxybenzaldehyde	8.36	164	0.18	164(23),121 µ+ (100%),10(2),93(10),77(8),65(19),51(11),39(24),27(24), 15(2).	
16	Phenol, 3-(dimethylamino)-	8.44	137	0.76	137 µ+ (100%),121(11),103(6),94(8),77(1),65(11),51(1),39(11)27(2), 15 (3).	
17	2,4-Hexadiene, 2,3,4,5-tetramethyl	8.778	138	0.23	138(19),123 µ+ (100%),108(7),95 (22),81(54),67(22),55(29),91(24).	
18	Acetic acid, 6,6-dimethyl-2-methylene-7-(3-oxobutylidene)oxepan-3-ylmethyl ester	9.087	248	0.30	196(4),150(1),137 µ+ (100%),109 (10), 94(14),71((22),57(8),42(39).	
19	4-((1E)-3-Hydroxy-1-propenyl)-2-	9.996	180	0.46	180(69),165(3),152(93),137 µ+ (100%),124(18),109(59),91(40),7764(21),51(21),29(13),15(10).	

	methoxyphenol					
20	1-Bromodocosane	10.139	249	0.36	309(6),169(4),135(46),85(47),57µ+ (100),29(11).	
21	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	10.386	212	1.01	212 µ+ (100),197(13),181(99),153 (13),138(12),123(8),108(5),93(5),67 (9),39(9),15(8).	
22	2-Chloropropionic acid, hexadecyl ester	10.8	332	0.44	269(4),222(6),196(11),168(3),139(6),111(56),83(98),57 µ+ (100),29(11).	
23	Octadecane	10.98	254	1.49	141(3),113(9),85(51),57 29(11).	
24	Benzene, 1,4-dimethoxy-2-methyl-	11.06	152	0.79	152(65),137 µ+ (100),109(12),94(9),77(12),66(6).	
25	Benzeneacetic acid, .alpha.-hydroxy-4methoxy-, methyl ester	11.227	196	1.38	196(11),151(6),137 µ+ (100),109 (19),94(15),77(19),63(6),39(7),15(8).	
26	Phenol, 2-methoxy-4-(1-propenyl)-,acetate	11.75	206	0.60	206(4),164 µ+ (100),149(25),131 (12),104(4),91(7),77(12),63(4),43(16).	
27	6-Octen-1-ol, 3,7-dimethyl-,acetate	11.833	198	0.21	138(39),123(56),109(27),95(76),81(81),67(76),43 µ+ (100),29(12),15(4).	
28	1,2-Pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-	11.97	353	0.33	366(3),203(18),175(24),149(25),123 (73),97(66),71 µ+ (100),43(91).	

	tetramethyl-1-naphthalenyl)-3-methylene					
29	Tridecanedial	12.46	212	0.15	213(3),194(2),176(3),150(19),124(38), 109(43),95(99),81(79),55 µ+ (100),41(83),27(42).	
30	Heptacosyl acetate	12.95	438	0.10	139(77),97(9),43 µ+ (100).	
31	Dotriacontyl heptafluorobutyrate	13.03	662	0.11	167(2),111(59),57 µ+ (100).	
32	Octacosyl heptafluorobutyrate	13.309	606	0.08	139(11),97(63),57 µ+ (100).	
33	Tricosane	13.621	324	0.11	324(12),253(3),225(3),197 (4),169(5),141(6),113(7),85(8), 57µ+ (100).	
34	Hexadecanoic acid, methyl ester	14.196	268	0.43	270(15),227(12),185(7),143(20), 115(3),97(7),74 µ+ (100),43(42).	
35	Dodecane, 1,12-dibromo-	14.563	328	0.09	193(6),177(6),137(27),111(10), 83(27), 55 µ+ (100),39(77).	
36	Ethanol, 2-(octadecyloxy)-	14.68	314	0.16	283(5),252(4),224(4),141(5),111(16), 85(46),57 µ+ (100).	
37	n-Hexadecanoic acid	15.641	256	4.80	256(17),213(17),185(11)157(14),129 (45),101(21),83(27),60 µ+ (100),43(84).	
38	Pentadecyl heptafluorobutyrate	16.312	255	0.65	255(2),210(7),169(12),125(16), 97(72),57µ+ (100)29(7).	
39	Eicosane	14.54	282	1.6	282(3),197(2),169(3),141(6),113(14), 85(63),57 µ+ (100).	

antibacterial inhibition against pathogenic bacteria were obtained in the extracts of plant materials measured growth inhibition zone ranged from 1 to 18 mm for all the sensitive bacteria. Table (2) indicated that, *Pimpinella* *anisum* extracts which prepared by petroleum ether exhibited higher activity against *B. cereus* (15 mm). On the other hand, chloroform exhibited higher activity against *P. aeruginosa* (18 mm). Finally, ETOAC extract exhibited higher antibacterial activity against *E. coli* (11 mm), while it was completely devoid of any activity against *K. pneumonia*. Increasing the extracts concentration of *Pimpinella anisum* led to progressive inhibition of tested bacteria, the highest value was recorded at (50 mg/ml) as shown in Figure (1). This possibly occurred due to natural bioactive compounds in *Pimpinella anisum*, which can used for medicinal purposes according to [3].

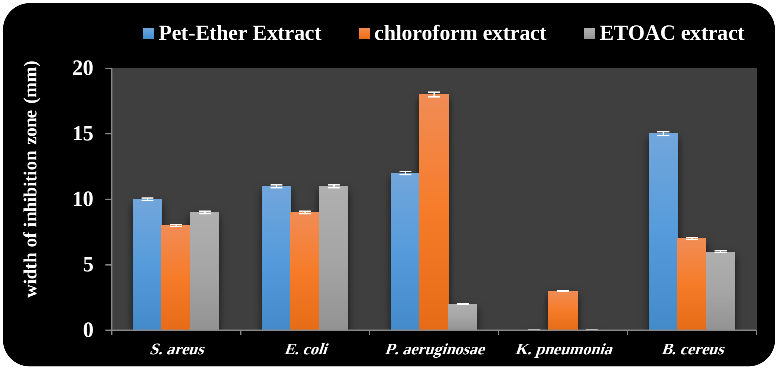


Figure (1). Antibacterial activities of different extracts at concentration 50 mg/ml from *Pimpinella anisum*,

Table (2). Antibacterial activities of *Pimpinella anisum* extracts against tested bacterial organism (mean±SD)

Antibacterial activity Compound	Concentration (mg/ml)	width of inhibition zone (mm)				
		S. areus	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*B. cereus*
1- *Pimpinella anisum* Pet-Ether Extract	1	2 ± 0.12	3 ± 0.12	5 ± 0.29	0	5 ± 0.29
	10	6 ± 0.29	5 ± 0.29	8 ± 0.58	0	7 ± 0.58
	25	8 ± 0.058	8 ± 0.58	10 ± 0.58	0	9 ± 0.58
	50	10 ± 0.058	11 ± 0.58	12 ± 0.58	0	15 ± 0.58

2- ***Pimpinella anisum*** **chloroform extract**	**1**	**2 ± 0.06**	**2 ± 0.06**	**8 ± 0.29**	**0**	**3 ± 0.12**
	10	**5 ± 0.12**	**4 ± 0.17**	**10 0.58±**	**0**	**5 ± 0.12**
	25	**6 ± 0.29**	**7 ± 0.29**	**15 ± 0.58**	**2 ± 0.06**	**6 ± 0.17**
	50	**8 ± 0.058**	**9 ± 0.58**	**18 ± 0.58**	**3 ± 0.12**	**7 ± 0.58**
	1	**1 ± 0**	**3 ± 0.12**	**0**	**0**	**2 ± 0.06**

The data represented in Table (2) and Figure (1) indicated that antibacterial activities of all extracts increased linearly with an increase in the concentration of extracts (mg/ml). These results are in agreement with [4].

The effect of the main factors (concentrations and extracts) and their interaction on antibacterial activity of *Pimpinella anisum*, extracts were very highly significant ($p<0.05$) as shown in Table (3). The effect of extracts were stronger (with a higher F ratio) than that of a concentration for all bacterial species. Figure (2) indicated the arrangements of the effective bacterial species in all extracts of *Pimpinella anisum*, *B. cereus* was the most effective bacterial species then *P. aeruginosa*, where *K. pneumonia* was the least one.

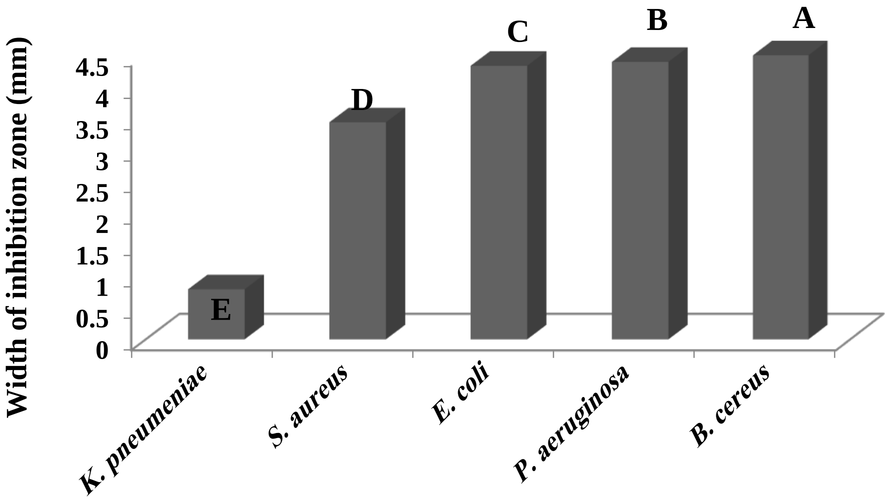


Figure (2). Different letters denotes significant differences based on two way ANOVA analysis of plants extracts where a>b.

Table (3). Two-way ANOVA showing the effect of the main factors (concentrations and extracts) and their interaction on bacterial inhibition zone diameter of plants extracts

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Plant	2933.8	3	977.9	1255.3	.000
Extracts	2229.2	2	1114.6	1430.8	.000
Conc.	1068.8	3	356.3	457.3	.000
Bacteria	1429.3	4	357.3	458.7	.000
Plant * Extracts	970.7	6	161.8	207.7	.000
Plant * Conc	354.9	9	39.4	50.6	.000
Plant * bacteria	970.2	12	80.9	103.8	.000
Extracts * Conc	115.7	6	19.3	24.8	.000
Extracts * bacteria	755.8	8	94.5	121.3	.000
Conc * bacteria	150.2	12	12.5	16.1	.000
Plant * Extracts * Conc	51.2	18	2.8	3.7	.000

Plant * Extracts * bacteria	1206.3	22	54.8	70.4	.000
Plant * Conc * bacteria	124.0	36	3.4	4.4	.000
Extracts * Conc * bacteria	76.1	24	3.2	4.1	.000
Plant * Extracts * Conc * bacteria	180.6	66	2.7	3.5	.000
Error	380.2	488	0.8		

Antifungal activity screening

Chloroform extract and ETOAC extract of *Pimpinella anisum* exhibited more activity against *Alt. alternata* as compared to petroleum ether extract. ETOAC extract showed more activity against *P. notatum* (1-6 mm). The results also showed that the higher concentration of ETOAC extract, and the greater effect on the *P. notatum* growth inhibition occurred, these agreeable with the finding reported by [5]**.** In contrast, petroleum ether extract showed any activity against the tested fungus in all concentration

Table (4). Antifungal activities of different plant xtracts against tested fungal organism (mean±SD)

Compound	**Concentration (mg/ml)**	**Antifungal activity width of inhibition zone (mm)**				
		Candida albicans	***P. notatum***	***Alt. alternata***	***A. niger***	***F. oxysporium***
Pimpinella anisum **Pet-Ether Extract**	**1**	**0**	**0**	**0**	**0**	**0**
	10	**0**	**0**	**0**	**0**	**0**
	25	**0**	**0**	**0**	**0**	**0**
	50	**0**	**0**	**0**	**0**	**0**
Pimpinella anisum **Chloroform extract**	**1**	**0**	**0**	**5 ±0.2**	**0**	**0**
	10	**0**	**0**	**8 ±0.1**	**0**	**0**
	25	**0**	**0**	**10 ±0.17**	**0**	**0**
	50	**0**	**0**	**11 ±0.06**	**0**	**0**
Pimpinella anisum **ETOAC extract**	**1**	**0**	**1 ±0.06**	**0**	**0**	**0**
	10	**0**	**2 ±0.06**	**0**	**0**	**0**
	25	**0**	**5 ±0.12**	**1 ±0.06**	**0**	**0**
	50	**0**	**6 ±0.06**	**2 ±0.06**	**0**	**0**

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