Antibacterial activity of Thymus vulgaris and Prunus amygdalus extracts against bacterial wound infection

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الخلاصة:

جمعت 80 مسحة سريرية من مرضى مراجعين لمستشفى مدينة الطب يعانون من التهابات الجروح، وقد تم تشخيص 67 عزله مسببة للمرض وحسب النسب المؤية المشار أزاء كل منها: Klebsiella spp. (22.4%)، Escherichia coli (22.4%)، Pseudomonas spp. (18%)، Staphylococcus aureus (15%)، Enterobacter spp. (10.4%)، Proteus spp (7.5%)، Streptococcus spp. (3%) و Acinetobacter spp. (1.5%).

تم دراسة فاعلية 11 مضادا حيوي ضد البكتريا المشخصة، حيث أبدت العزلات تحسسا للامبنيم وبنسبة 97% يليه الاميكاسين بنسبة 89.5% فيما كانت معظم العزلات مقاومة للمضادات سيفوتاكسيم، توبرامايسين، تتراسايكلين، ببراسلين والجنتامايسين وبنسبة 89.5%، 82%، 82%، 77.6% و77.6% على التوالي، كما أظهرت جميع العزلات الموجبة لصبغة كرام حساسية للفانكومايسين وبنسبة 100%.

تمت دراسة فاعلية المستخلص الهكساني للزعتر Thymus vulgaris واللوز الحلو Prunus amygdalus ضد العزلات متعددة المقاومة للمضادات الحيوية من خلال استخدام طريقة الانتشار بالاكار agar-well diffusion وعند التراكيز 1:2, 1:4, 1:8, 1:16, 1:32.

اظهرت النتائج أن مستخلص الزعتر الهكساني (thyme) له فاعلية قتل ضد بكتريا Acinetobacter وبكتريا *E. coli* فقط عند التراكيز (1:2, 1:4) حيث كان قطر التثبيط (8mm, 12mm). اما بكتريا Pseudomonas spp. ، Staphylococcus aureus و Streptococcus spp. فلم تظهر اي تحسس تجاه التراكيز المستخدمة لمستخلص الزعتر الهكساني.

أظهر مستخلص اللوز الحلو الهيكساني (almond) أفضل تأثير على بكتريا Klebsilla spp., Enterobacter spp. و Proteus spp. ولكافة التراكيز. أما Pseudomonas spp. و *E. coli* فقد أبدت تحسسها للتراكيز (1:2,1:4,1:8) في حين اظهرت بكتريا Streptococcus spp. و Staphylococcus aureus حسساسيتها للمستخلص عند التراكيز (1:4 و1:2) حيث كانت مناطق تثبيط النمو لكلا الجنسين (8mm و 7mm) و (10mmو9mm) على التوالي.

Abstract:

Eighty clinical swabs were collected from Patients suffering from wound infection, attending medical city hospital Sixty seven isolates were diagnosed as causative agents, they were Klebsiella spp. (22.4 %), Escherichia coli (22.4%), Pseudomonas spp. (18%), Staphylolcoccus aureus(15%),Enterobacter spp(10.4%), Proteus spp (7.5%) Streptococcus spp (3%) and Acinetobacter spp.(1.5%); Eleven antibiotics were used for suscpitbility test of drugs. Most of isolates were sensitive to lmipenem in percentage 97% and Amikacin in percentage of 89.5% while most isolate were resistance for many antibiotics like Cefotaxim (89.5%), Tetracyclin (82%), Tobramycin (77.6%), Pipracilin (77.6%) and Gentamycin (77.6%), isolates from gram positive bacteria showed sensitivity (100%) for Vancomycin .

Antimicrobial activity of Thymus vulgaris and prunus amygdalus against higher resistance isolates was estimated, using two folds dilution extracts in agar diffusion technique at concentrations of 1:2, 1:4 , 1:8, 1:16, 1:32 .Thymus vulgaris has shown anti bacterial activity against Acinetobacter spp., proteus spp. And Klebsiella spp.in all concentration were used .The lowest effect was on E.coli (12 mm, 8 mm) at concentration 1:2, 1:4. No effect was clear on Pseudomonas spp., Staphylococus aureus and Streptococcus spp .

Hexane extract of Sweet almond extract showed the best effect on Klebsiella spp.,Enterobacter spp., Proteus spp. at all concentrations used, while E.coli and pseudomonas spp showed sensitivity concentrations of 1:2, 1:4, 1:8, the activity on Gram positive bacteria :Staphylococus aureus and Straptococus spp. was at concentrations of 1:2 , 1:4 by measuring inhibition zone which was (8mm,7mm) and (10mm,9mm) respectively.

Keywards: Thymus vulgaris, prunus amygdalus, hexane extract, wound infection.

Introduction:

Pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased [1]. The increasing failure of chemo therapeutics and antibiotics resistance exhibited by pathogenic microbial infectious agents had led to the screening of several medicinal plants for their potential antimicrobial activity [2].

plants have given western pharmacopoeia have given about 7000 different pharmaceutically important compounds and a number of top selling drugs of modern time,e.g. Quinine, artemisinin, taxol and Camptothecin[3].

Almond (Prunus amygdalus belong to Rosaceae) is an important food crop, varying in use from local consumption as an edible nut in its natural state to inclusion as a major ingredient in manufactured food products , the nutritional importance of almond fruit is related to its Kernal, which is concentrated as a

source of energy, supplying significant amounts of fats, protein and fiber [4]. Almond also include considerably macronutrients and micronutrients [5], and is a good, source of nutrients associated with heart health such as vit. E mono unsaturated fatty acids, arginine and potassium [6]. Almond has antioxidant activity [7], antibacterial, antifungal activity [8] and Almond is used in cosmetics as skin care products [9].

Thyme (Thymus vulgaris, belong to Lamiaceae), has a very long history of folk use for a wide range of ailments it is an excellent antiseptic and due to the presence of phenolic compounds, thymol and carvacrol [10]. Thyme phytochemicals have been used as antioxidant [11], antibactrial and antifungal[12,13], antiprotozoal [14], and in wound healing [15].

The aim of this study was to determine the antibacterial activity of hexane extract of prunus amygdalus and thymus vulgaris against wound pathogenic bacteria.

Materials and Methods:

Specimen Collection:

Eighty swab of wound infection were collected from patients attended medical city hospital in Baghdad during January till March 2008.

Microorgansims:

The typical microorganisms were obtained after culturing the wound swabs on blood agar & macconkey agar (oxoid). Biochemical test (Indol, Tsi, Urea, Simmon citrate and semi solid manitol), suspected colony (Api 20, Biomereux, France) for gram negative bacteria. Manitol salt agar, coagulase and catalase test for S.aureus with gram stain for all isolates [16].

Antibiotic susceptibility test:

Susceptibility test of the isolate was done using the Kirby-Bauer disk diffusion method by using Mueller – Hinton agar, the agar plate left at room temperature for 1h to allow diffusion of the antibiotics into the agar medium. Plates were incubated at 35 – 37 $C^{o}$for 24h , zone of growth inhibition was then measured to the nearest millimeter and recorded (17).The antibiotics disks used were Imipenem (Imp), Amikacin (AK), Ciprofloxacin (Cip), Cefotaxim (CTX), pipracillin (Pip), Gentamycin (CN), Tobramycin (Tb), Augmentin (Aug), Tetracyclin (TE), Erythromycin (E) and Vancomycin (VA). Isolates were classified as either resistant or inter mediate or sensitive based on definition of the clinical and Laboratory standard Institute of WHO .Resistant and intermediate isolate were grouped together for analysis in this study. An isolate was considered multiresistant if it was resistant to at least three of the antibiotics tested , quality control on the susceptibility disk were prformed using laboratory strains E.coli , P.aeruginosa& S. aureus .

Prepartion of extracts:

The seeds samples of Almound & Thyme were collected from herbal shops Almound&thyme seeds were milled by using blender, the resulting powder was kept. The extraction was carried out by Soxhlet extraction method using-hexan as solvent Thirty grams of seeds powder was extracted with 300ml of solvent. By using soxhlet appartus for 10h at temepratur not exceeding the boiling point of the solvent, then the extract concentrated under vacum at 40-50^{o}C by using a rotary evaporator [19]. The consternations 1:2, 1:4, 1:8, 1:16, 1:32 were made by dissolving extract in DMSO (V/V).

Antibacterial assay:

According to [8] The antibacterial activity was evaluated on multi drug resistant bacteria using agar well diffnsion Method, using Mueller–Hinton agar (oxoid) for bacteria were selected from 18-24h, turbidity was visually adjusted to that of 0.5 McFarland turbidity standard (1.5 X 10^{8}CFU/ml).The inoculums were swabbed on to the surface of agar plates with sterile cotton swab. Six-millimeter wells were punched in to the agar and filled with half dilution of seeds extracts (1:2, 1:4, 1:8, 1:16, 1:32) V/V by transferred to wells using micropipette, DMSO used as negative control. The plates were kept in the refrigerator for 3-5 mint for diffusion and incubated at 37^{o}C for 24h, inhibition zone was measured in millimeter [20,21].

Results and Discussion:

Sixty seven isolates were diagnosed as causative bacteria as shown in (Table-1). The most causative agent was Klebsiella spp. and Escherichia coli in Percentage 22.4% for each one, followed by pseudomonas spp.(18%), S. aureus(15%), Enterobacter spp. (10.4%), Proteus spp. (7.5%), Streptococcus spp.(3%), while the lowest causative agent was Acinetobacter spp. in percentage of 1.5%.

Bacterial isolates	**No.**	**(%)**
Escherichia coli	15	22.4
Klebsiella spp.	15	22.4
Pseudomonas spp.	12	18
Staphyloccus aureus	10	15
Enterobacter spp.	7	10.4
Proteus spp.	5	7.5
Streptococcus spp.	2	3
Acinetobacter spp	1	1.5
Total	67	

Table-1: percentage of Bacterial isolate caused wound infection.

Studies showed the following microorganisms act as causative agent of wound infection: S. aureus (17%), Enterococcci (13%), coagulase negative Staphylococcus (12%) Escharichia coli (10%), Pseudomonas spp. (8%) Enterobacter spp. (8%) proteus mirabilis (4%), Klebsiella pneumonia (3%), and Candida albicans in percentage 2% [22,23].

The sensitivity of isolated bacteria to antibiotics was shown in (Table-2) the more effective antibiotic was Imipenem, the isolates showed sensitivity in percentage of 97% followed by Amikacin, Ciprofloxacin, pipracyllin, Gentamycin, Augmentin, Tetrcayclin, tobramycin, Erythromycin and cefotaxin.

Bacterial isolates	IMP (10) mg	AK (15)mg	CIP (10)mg	PIP (100) mg	CN (30)mg	AUg (10)mg	TE (30) mg	TB (10)mg	CTX (10)mg	E (30)mg	V (25)mg
E. coli	0	1	7	12	12	10	13	12	13	-	-
Klebsiella spp.	0	1	7	11	12	12	11	13	13	-	-
Pseudomonas spp.	2	1	6	7	9	11	11	9	11	-	-
Staph. aureus	-	2	3	10	8	7	9	10	9	4	0
Enterobacter spp.	-	-	4	5	5	7	6	4	6	-	-
Proteus spp.	-	-	2	4	3	4	2	4	5	-	-
Streptococcus spp.	-	-	-	2	2	1	2	2	2	2	0
Acinetobacter spp	-	1	1	1	1	1	1	1	1	-	-
Total of Resistant isolates	2 (3%)	7 (10.4%)	30 (44.8%)	52 (77.6%)	52 (77.6%)	52 (77.6%)	55 (82%)	55 (82%)	60 (89.5%)	6 (50%)	0 (0%)

Table-2: Number and Percentage of Resistant bacterial isolates.

The antimicrobial properties of planties of plants have been investigated by a number of researchers worldwide [24]. The nature and number active antibacterial principles involved in Thymus vulgaris and Prunus amygdalus extracts of our study are not investigated but the results showed the antibacterial activity of those extracts against multi drug resistant pathogenic bacteria, as shown in (Table-3) and (Table-4).

The results of antibacterial activity of Thymus vulgaris seeds extracts in concentrations of 1:2, 1:4, 1:8, 1:16, 1:32 were shown in (Table-3). The highest effect was on Proteus spp., Klebsilla spp. and Acinetobacter spp. at all concentrations used with different inhibition zone diameters. Enterobacter spp. appeared inhibition zones 13mm, 12mm, 11mm, 8mm at concentrations of 1:2, 1:4, 1:8, 1:16 respectively. The lowest inhibition zone was on Escherichia coli

which was 12mm, 8mm at concentrations of 1:2, 1:4, respectively. No inhibition was against Pseudomonas spp. and Gram positive bacteria: S. aureus and Streptococcus spp.

The strongly antiseptic and antifungal activity of thyme is mainly due to the presence of phenolic compounds, thymol and carvacrol (10), the content of thymol in thyme essential oil is much higer compared to carvaerol content [25].

Bacterial isolate	Inhibition zone(mm) in diameter					
	1:2	1:4	1:8	1:16	1:32	Control (DMSO)
Escherchia coli	12	8	-	-	-	-
Klebsiella spp.	13	12	11	10	8	-
Pseudomonas spp.	-	-	-	-	-	-
Staphyloccus aureus	-	-	-	-	-	-
Enterobacter spp.	13	12	11	8	-	-
Proteus spp.	13	12	11	10	9	-
Streptococcus spp.	-	-	-	-	-	-
Acinetobacter spp.	13	12	10	9	7	-

Table -3: Antbacterial activity of hexane extract of Thymus valyaris seeds.

(Table-4) showed the antibacterial activity of hexane extract of prunus amygdalus on isolated multidrug resistant bacteria, Klebsiella spp., Proteus spp. and Enterobacter spp. showed sensitivity to all concentrations used with differences in inhibition zone diameters. Escherichia coli, Pseudomonas spp. and Acinetobacter spp. were sensitive in concentrations 1:2, 1:4, 1:8, almond extract have shown activity against Gram positive bacteria, The highest effective was on Streptococcus spp. It was 10mm, 9mm at concentration of 1:2, 1:4 respectively while Inhibition zone was 8mm, 7mm for S. aureus. The activity of almond maybe due to its content of oils[21], flavonoids and phenols [6].

Bacterial isolate	Inhibition zone(mm) in diameter					
	1:2	1:4	1:8	1:16	1:32	Control (DMSO)
Escherchia coli	9	8	8	-	-	-
Klebsiella spp.	15	13	11	10	8	-
Pseudomonas spp.	12	11	10	-	-	-
Staphyloccus aureus	8	7	-	-	-	-
Enterobacter spp.	13	12	11	10	10	-
Proteus spp.	12	11	10	8	8	-
Streptococcus spp.	10	9	-	-	-	-
Acinetobacter spp.	14	10	10	-	-	-

Table-4: Antibacterial activity of hexane extracts of prunus amygdalus seeds.

The mechanisms of action of antimicrobial effects is due to active principles which toxic to microbial pathogens or they may be impair variety of enzyme systems including those involved in energy production and structural component synthesis. Conclusively, plants are evaluable sources for new compounds and should receive special attention in research strategies to develop new antimicrobial agents**.**

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