

In vitro : Antimicrobial activity of *Matricaria chamomilla* oil extract on clinical isolates of *Pseudomonas aeruginosa*⁺

مختبرياً: التأثير الضد مايكروبي للمستخلص الزيتي لنبات البابونك ضد جراثيم الزائفة الزنجارية المعزولة سريراً

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Abstract :

The recent study comprised isolation and identification of *Pseudomonas aeruginosa* from 50 burn swabs samples of in and out patients of AL – Sadr , AL – Karama and Baghdad hospitals in Baghdad city with 20 , 15 and 15 samples respectively .

The results showed 11 , 2 and 5 of pure isolates of *Pseudomonas aeruginosa* were obtained with total percentage 36% . Also the results explained the higher activity of *Matricaria chamomilla* oil extract on bacterial growth which rang from 90 to 100% , by 12 – 19 mm zone of inhibition to *Pseudomonas aeruginosa* isolates .

These activities of oil extract were due to its chemical structure and their synergetic effects .

For the medical value and reducing of bacterial resistance , the study recommended to done more researches in the same subject and having new results that may by used in medical treatments .

الخلاصة :

تضمنت الدراسة عزل وتشخيص جراثيم الزائفة الزنجارية *Pseudomonas aeruginosa* من (٥٠) عينة لمسحات الحروق من المرضى الراقدين والمراجعين في ثلاث مستشفيات الصدر، الكرامة، بغداد في مدينة بغداد وبواقع (٢٠، ١٥، ١٥) عينة من كل مستشفى على التوالي .

توزعت نتائج العزل و التشخيص بين المستشفيات بواقع (١١، ٢، ٥) عزلة نقية لجرثومة الدراسة وللمستشفيات الثلاث على التوالي وبنسبة إصابة إجمالي (٣٦%) .

كذلك أشارت نتائج الدراسة إلى فعالية المستخلص الزيتي لنبات البابونك ضد عزلات جرثومية الدراسة وبنسبة حساسية عالية جداً تراوحت ما بين (٩٠ – ١٠٠%) تبعا لمصدر العزلة ، كانت معدل التثبيت للنشاط الضد مايكروبي للمستخلص يتراوح ما بين (١٢ - ١٩ ملم) .

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توصي الدراسة استمرارية البحث العلمي الدقيق من أجل الاستخدام العلمي والطبي الأمثل لمستخلصات النباتات الطبية الموجودة في بلدنا والاعتماد علميا في علاج المسببات الجرثومية المختلفة لما لها من دور كبير بالتقليل من مخاطر المقاومة الجرثومية للمضادات المستخدمة طبيا في الوقت الحاضر .

Introduction :

Pseudomonas aeruginosa is a gram – negative bacillus , non – spore forming , non capsulate and usually motile by virtue of one or two polar flagella [1] . It is a strict aerobe and grows readily on a wide variety of culture media over a wide range of temperature through emits a sweet grapelike odour that is easily recognized [2] . The organism produces diffusible pigments ; typically , the colony and surrounding media is greenish – blue on cetrimide agar due to production of a soluble blue phenazine pigment [3] .

Pseudomonal infection in hospital patients is usually localized , as in catheter – related urinary tract infection , infected ulcers , bed sores , burns and eye infections [4] . Recent clinical data indicates Pseudomonas aeruginosa to be the fourth leading cause nosocomial infection and the foremost cause of hospital – acquired pneumonia [5] . Acquisition of this pathogen within the hospital setting is attributed to contaminated environmental reservoirs , as well as patient to patient spread [6] .

Pseudomonas aeruginosa is normally resistant to many commonly employed antimicrobial agents which occur as a result of low permeability of the outer membrane [7] , combined with the presence of both Beta – lactamases and multidrug efflux pumps [8] . With the development of antipseudomonal Beta – lactam compounds , including piperacillin , ticarcillin , ceftazidime meropenem and combination of an aminoglycosides such as gentamicin or tobramycin was commonly adopted [9] .

Oil extract of many plants having medical value especially those on pathogenic agents that contaminate wound and burns [10] .

Matricaria chamomilla oil extract had a wide antimicrobial activity on Pseudomonas aeruginosa , Streptococcus pyogenes , Staphylococcus aureus and Escherichia coli [11] . These activities are due to the chemical composition that found in its structure such as triterpens or sterols , triterpenoids and phenolic components [11 , 12] .

Aims of the Study :

1. Isolation and identification of Pseudomonas aeruginosa from clinical isolates [burns] from three hospitals in Baghdad city .
2. Extraction , purification and characterization of Matricaria chamomilla oil extract from local plants .

3. Studying the antimicrobial activity of Matricaria chamomilla oil extract against Pseudomonas aeruginosa isolates in vitro .

Patients and methods :

1. Patients :

Fifty samples were collected from adult patient [women and men] suffering from burns with different stages . The period was from January to March 2006 . Patients were taken from in and outpatients admitted to AL- Sader hospital . Al- Karama hospital and Baghdad hospital .

2. Collection of specimens :

The specimens were generally collected from patients with burns .The swabs are inserted in to the upper part of the skin under septic condition . The swabs were collected by sterile swabs and then placed in sterile tubes containing normal saline to maintain the swabs moist until taken to laboratory the swabs had been inoculated on Mac Conkey agar and incubated aerobically for 24hr. At 37°C temperature [13] .

3. Isolation and identification :

Fifty Swabs were collected ,then cultured on nutrient agar Blood agar and Mac Conkey agar . Then the selected colonies from Mac Conkey agar were cultured on Blood agar again which incubated overnight at 37°C for demonstrated their ability for Blood haemolysis [14] . After cultured the selected colonies on 0.03% cetrimide agar [15] , Pseudomonas aeruginosa isolates were obtained that used for biochemical tests [16] .

4. Pure isolates :

The study was obtained separated and pure isolates of Pseudomonas aeruginosa after checking the following tests :

A. Macroscopic appearance of colonies .

B. Microscopic appearance of colonies .

C. Biochemical test such as :

1- Oxidase test .

2- Catalase test .

3- Urease test .

4- Citrate utilization .

5- Voges proskauer test .

6- Methyl red test .

7- H₂S production test

8- Indol production test.

All these test were done according to [17] .

5. Oil extraction of Metrication chamomilla obtained by :

a. The plant classified by Agriculture college of Baghdad University as a genus of the family composite .

b. 50 gm plant put thimble and by soxhlet continuous extraction for 16-24 hrs . With 250ml of n – hexam to have a solution , that being used by rotary evaporator in 40°C to have the final thick oil [18] .

c. The percentage of oil was calculated according [19] .

$$\text{Oil \%} = \frac{\text{Oil obtained}}{\text{Plant weight}} \times 100$$

6. Sensitivity test used for detection of extract on bacterial growth according to agar – well diffusion method [20] ,by using Four Well in each plate and the volum of extracted oil was 0.01 ml which put in the separated Well. The inoculated plate was incubated at 37C for 18-24 hours (20).

Results :

Out of 50 samples of burned patients collected from three hospitals of Baghada city . 18 isolates –were diabnosed as Pseudomonas aeruginosa though macroscopic of colonies and microscopic finding of gram stain , which is gram negative bacilli , non sporing and non capsulated .

Standard biochemical tests detected Pseudomonas aeruginosa isolates through the results which listed in table (1) .

Table (1) : Results of biochemical test of Pseudomonas aeruginosa

Brochemical tests	Result
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Oxidase	+
Catalase	+
Urease	+ (variable)
Citrate utilization	-
Voges proskuor	-
Methyl red	-
H ₂ S Production	-
Indol production	-
Nitrate reduction	+

Primary isolated of Pseudomonas aeruginosa wave performed by culturing each one on MacConkey's agar ,pseudomonad colonies showed non laetose fermenter (Pale appearance) . Selected colonies also cultured on blood agar which get clear beta hemolytic appearance . After that all the microscopic examination were successfully diagnosed to Pseudomonas aeruginosa .

Table (2) explained the distribution of Pseudomonas aeruginosa isolates from their sources , the highest percentage of isolates were appeared in Al – Sadr hospital as shown in the table .

Table (2) : Distribution of Pseudomonas aeruginosa according the source of sample

Source(2) sample	Total sample	<u>Ps. aeruginosa</u>	%
Al - Sadr Hospital	20	11	55
Al – Karama hospital	15	2	13
Baghdad hospital	15	5	33

By using Soxhlet continous extraction the study obtained 9.3% oil extract of Matricaria chamomilla , which the chemical appearance showed that its composition

contain carbohydrates , triterpens , triterpenoids , tannis , glycosides and phenolic compounds with different concentrations .

The results showed that 90% , 100% , 100% , of *Pseudomonas aeruginosa* isolates were sensitive to *Matricaria chamomilla* oil extract from Al-Sadr , AI- Karama Baghdad hospitals respectively . The zone of inhibition of in diameter these activities were ranged from 12-19 mm in diameter as shown in table (3) .

Table (3) : *Matricaria Chamomilla* activity on *Pseudomonas aeruginosa* isolates .

Source of isolates	No. Of isolates	Sensitive isolates		Zone of inhibition mm.	
		No.	%	Min.	Max.
Al - Sadr Hospital	11	10	90	13	17
Al – Karama hospital	2	2	100	15	19
Baghdad hospital	5	5	100	12	17
Total	18	17	94.4	12	19

DISCUSSION :

In the recent study , 50 burn swabs obtained from in and outpatients whom suffering from burn . these samples admitted to three hospital in Baghdad city for the period January to March 2006 .

All burn swabs were subjected for culturing on available media and it was found out of total 50 samples , there were only 18 bacterial isolates of Pseudomonas aeruginosa no growth was seen in other samples (32 samples) which would indicate other pathogenic agents because of bacterial contamination to the outer layer of the burns ,or because of antibacterial effects that used [21] .

The percentage (36%) of Pseudomonas aeruginosa isolates from the total patient sample's , indicate the spreading severity of this bacteria among those patients specially the burns [22] , while the differentiation of isolates from the three sources were because of the healthy care of each area .

Using of 0.03% cetrimide agar in the pure culture of Pseudomonas aeruginosa was because of containing citrimethyl ammonium bromide which inhibited all other members of the genus Pseudomonas , and could obtained green pigment and other characteristic odor [16,17] .

The results of Matricaria chamomilla oil extract that obtained by [23, 24] whom have pointed that this oil extract have different compounds such as triterpens , triterpenoids , tannins glycosides and phenol , while these compound were having antibacterial activity of several bacterial growth specially on Pseudomonas aeruginosa [25,26,27] .

Antimicrobial activity of Matricaria chamomilla oil extract was investigated against 18 isolates of Pseudomonas aeruginosa , 17 isolates were sensitive with percentage that shown in table -3- . This results explained the high medical activity (value) of the oil extract against the bacterial study , which is identical with those obtained by other researcher [28,29] .Besides of the chemical extract component , the antimicrobial activity might increase due to its active groups which found and cause the synergistic effects on the Pseudomonal growth [30] .

Because of bacterial resistance to several types of antibiotics and medical value(activities) that found in the study , the study recommended to spread of medical plants in treatment of different kinds of pathogenic microorganisms.

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