Antibacterial effect of phenolic extracts of *Plantago lanceolata, Convolvulus arvensis* and *Euphorbia granulate* leaves against human pathogenic bacteria.

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التأثيرات المضادة للبكتريا للمستخلصات النباتية الفينولية لثلاث نباتات طبية محلية (لسان الحمل, المديد, سرطان الثيل) ضد بعض الجراثيم المرضية المصيبة للإنسان.

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المستخلص

الهدف من الدراسة هو تحديد الفعالية المضادة للبكتريا المرضية للمستخلصات الفينولية لأوراق ثلاثة نباتات طبية محلية وهي نبات لسان الحمل والمديد وسرطان الثيل, بتراكيز مختلفة (5 ملغم/مل , 10ملغم/مل , 20ملغم/مل) كجزء من البحث عن مواد جديدة ممكن استخدامها كعلاج ضد أهم المسببات المرضية للإنسان. حيث استخدمت هذه المستخلصات وبتراكيزها المختلفة ضد نمو أربعة جراثيم مرضية (اثنين من الجراثيم المرضية الموجبة لصبغة غرام واثنين من الجراثيم السالبة لصبغة غرام) باستخدام طريقة الانتشار في حفر الاكار من اجل اختبار الفعالية المضادة لهذه البكتريا ومقارنتها مع العلاجات المضادات الحيوية .

الكلمات المفتاحية: التأثير المضاد للبكتريا, المستخلص الفينولي , نبات لسان الحمل , نبات المديد, نبات سرطان الثيل.

Abstract

Antibacterial effects of phenolic extracts of three medical plants: *Plantago lanceolota*(Pl.); *Convolvulus arvensis*(Ca.) and *Euphorbia granulate*(Eg.) leaves, in different concentrations (5mg/ml, 10mg/ml and 20mg/ml) were carried out as a part of search for new antibacterial substance against important human pathogens. Antibacterial activities were carried out against four human pathogens, two Gram positive bacteria: *Bacillus subtilis*; *Staphylococcus aureus* and two Gram negative bacteria: *Pseudomonas aeroginosa*; *Klebsiella pneumonia*. By the standard procedures the zone of inhibition of the extract was compared with different standard antibiotic drugs like: Oxytetracycline, Streptomycin, Gentamycine, chloromphenicol and Ciprofloxacin. Observed results showed that phenolic extracted from three plants in a different concentrations possess effective antibacterial against all tested organisms.

Key words: Antibacterial effect, Phenolic extract, Plantago lanceolota, Convolvulus arvensis, Euphorbia granulata.

Introduction

Several antimicrobial drugs are available, but their use is limited by a number of factors, such as low potency, emergence of resistant strains and drug toxicity (1). The search for component with antimicrobial activity has gained increasing importance in recent times due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant microorganisms (2, 3). Jain S. *et al*, (4) recorded that plant provide an important source of natural products, many of them formed basis for the development of medicinally important drugs. Some plants are used in traditional medicine, about 75-80% of the world population, mainly in the developing countries, because of antimicrobial, antibacterial, antifungal and anti-inflammation properties with lesser side effects (5, 6). *Plantago lanceolota* (Pl.) one of the medical plants used to wound healing, draining abscesses, suppress cough and demonstrated good in vitro antimicrobial activity (7 , 8) . Although, Tokgun O. *et al*, (9), showed that *Convolvulus arvensis* (Ca.) possess different biological activities. It has been reported that several members of Convolvulaceae

family have antitumor activity against some tumor cell lines. Convolvulus arvensis have not been previously reported or have only very rarely been cited or indicated as plant foods in very restricted geographical area (10).While, *Euphorbia granulate* (Eg.), it is not extensively studied yet, no previous phytochemical and pharmacological investigation was conducted on this plant till now. The purpose of the present study was to investigate the antimicrobial activity of the phenolic extracts of three medicinal plants: PL. , Ca. and Eg. against the growth of four pathogenic bacteria.

Materials and methods

Plants: leaves of three medical plants: Pl., Ca. and Eg. were collected from different localities of Wasit gardens. The plants were identified in the Wasit University, College of Medicine, Department of Pharmacology and Toxicology.

Preparation of extracts: phenolic compounds were extracted according to Jeethu, (2), Ribereau-Gayon (11) and Harborne (12) Powdered plants were extracted with the organic solvents by soxhlet apparatus. The extracts were filtered and concentrated at 45 ^{0C} using rotator vacuum evaporator. Preparation of dilution of crude extract (5mg/ml, 10mg/ml and 10mg/ml) for antibacterial assay. Test for phenols according to Sameerah et al., (13) 2ml of the extract was stirred with 2ml of distilled water and few drops of ferric chloride solution (FeCl3)were added, the formation green precipitate was an indication for the presence of phenols.

Microorganisms: the microorganisms employed in the current study: two gram positive bacteria: *B.subtilis* and *S.aureus*. Beside two strains of gram negative bacteria including: *P.aeroginosa* and *K.pneumonia*. Were obtained from the laboratory of microbiology at the college of medicine Wasit University. These cultures of all organisms were inoculated in sterile nutrient broth at 37 ^{oC} and incubated till 0.5 Mc Farland Standard turbidity obtained, and then used for assay.

Sensitivity test: Antimicrobial assay was performed by agar well diffusion method. Muller Hinton medium used for antibacterial activity (4, 14).After complete solidification, four wells were made septically on the surface of each agar plate with a diameter of 5mm (with

exception of plates that were used for antibiotic study. Later, a sterile cotton swab was dipped into the nutrient broth culture suspension and the microorganism was striated in a plate. Finally and after the inoculums were dried, 0.1ml of each concentration of each extract was poured into the wells beside 0.1ml of 96% ethanol which considered as a negative control on the same extract plate. All the plates were incubated at 37 ^{oC} for 24-48 hours and following incubation, the diameter of zone of inhibition around each well was measured in millimeters. The antibacterial activities of the extracts were compared with standard antibiotics:Oxytetracycline,Streptomycin ,Gentamycin, Chloromphenicol and Ciprofloxacin.

Statistical Analysis:

The values are given as mean $\pm$ standard error of mean and the data were analyzed by ANOVA test with Least Significant differences (LSD) at significant level of ($P \leq 0.01$).

Results

All the phenolic plant extracts show antibacterial activity against human pathogenic bacteria: *B.subtilis*; *S.aureus*; *P.aeroginosa* and *K.pneumonia*, are presented in Table (1), (2) and (3). The antibacterial activity increased linearly with increase in concentration of extracts (mg/ml). the results revealed that among four pathogenic bacteria *B.subtilis* belong gram positive bacteria showed the higher susceptible for phenolic extract of Pl. at concentration 20mg/ml with mean zone of inhibition (1.916 $\pm$ 0.247 mm diameter).While the gram negative bacteria *K.pneumonia* showed least susceptible for Eg. Phenolic extract at concentration 20mg/ml with mean diameter zone of inhibition (0.633 $\pm$ 0.098 mm). From three Table noted that Ca. phenolic extract at concentration 5mg/ml recorded the least zone of inhibition (0.02 $\pm$ 0.018 mm diameter) against *S.aureus* .

The inhibitory effect of phenolic plant extracts with concentration (5mg/ml, 10mg/ml and 20mg/ml) showed in Figure:1, Figure: 2 and Figure: 3. As compared with standard drugs as a positive control (Table:4), Ciprofloxacin was the strongest among the used

antibiotics, it is produced significant inhibitory effect against the growth bacteria *B.subtilis*; *S.aureus*; *K.pneumonia* and *P.aeroginosa* with mean diameter zone of inhibition: 29.63mm, 27.5mm, 21.45mm and 16.3 mm respectively.While, Oxytetracycline gives the least zone of inhibition among used antibiotic against the above four bacteria with diameter 10.95mm, 12.05mm, 3.56mm and 12.63mm respectively, show (Figure: 4).

Table (1): Antibacterial activity of phenolic extract of *Plantago lanceolata* leaves against gram positive and gram negative bacteria.

Plant extr. / bacteria	Negative cont.	Pl. 5mg/ml	Pl. 10mg/ml	Pl. 20mg/ml
B. subtilis	0.438± 0.122 b	1.05 ± 0.187 b	1.4 ± 0.211 ab	1.916± 0.247 a
S.aureus	0.203 ± 0.11 b	0.633 ± 0.194 b	0.983± 0.233 ab	1.55± 0.331 a
P. aeroginosa	0.7 ± 0.126 b	0.85 ± 0.133 b	1.11 ± 0.177 ab	1.483± 0.234 a
K. pneumoniae	0.55 ± 0.138 b	0.56 ± 0.122 b	0.883± 0.144 b	1.516± 0.241 a

-The value represents diameter of the zone of inhibition (mm) Mean ±Standard Error

-The different small letters show significant effect ,while the same small letters show insignificant effect between different groups.

Table (2): Antibacterial activity of phenolic extract of *Convolvulus arvensis* leaves against gram positive and gram negative bacteria.

Plant extr. / bacteria	Negative cont.	Ca. 5mg/ml	Ca. 10mg/ml	Ca. 20mg/ml
B. subtilis	0.391± 0.09 b	0.408 ± 0.1003 b	0.783± 0.140 ab	1.1± 0.167 a
S.aureus	0.025 ±0.067 b	0.02 ± 0.0018 b	0.4± 0.093 b	1.183± 0.263 a
P. aeroginosa	0.616± 0.065 b	0.683 ± 0.047 b	0.783± 0.047 b	1.183± 0.070 a
K. pneumoniae	0.866± 0.08 b	0.75 ± 0.076 b	1.066± 0.095 b	1.533± 0.156 a

-The value represents diameter of the zone of inhibition (mm) Mean ±Standard Error

-The different small letters show significant effect ,while the same small letters show insignificant effect between different groups.

Table (3): Antibacterial activity of phenolic extract of *Euphorbia granulate* leaves against gram positive and gram negative bacteria.

Plant extr. / bacteria	Negative cont.	Eg. 5mg/ml	Eg. 10mg/ml	Eg. 20mg/ml
B. subtilis	0.041± 0.090 b	0.375 ± 0.085 b	0.611± 0.118 ab	0.95± 0.161 a
S.aureus	0.291 ±0.109 b	0.433 ± 0.071 b	0.616± 0.091 ab	0.916± 0.101 a
P. aeroginosa	0.35± 0.08 b	0.366 ± 0.105 b	0.633± 0.098 b	1.033± 0.133 a
K. pneumoniae	0.425± 0.087 b	0.508 ± 0.089 a	0.575± 0.101 a	0.633± 0.098 a

-The value represents diameter of the zone of inhibition (mm) Mean ±Standard Error

-The different small letters show significant effect ,while the same small letters show insignificant effect between different groups.

Table (4): Antibacterial activity of standard antibiotic (positive control) against gram positive and gram negative bacteria.

Bacteria / Standard antibiotic	B. subtilis	S. aureus	P. aeroginosa	K. pneumoniae
Oxytetracycline	10.95± 0.25	12.05 ± 0.48	3.56± 0.40	12.63± 1.03
Streptomycin	19.48 ±1.36	21.3 ± 1.50	4.93± 0.88	9.01± 1.23
Gentamycin	22.15± 1.87	20.13 ± 5.29	10.25± 0.88	17.85± 1.63
Chloromphenicol (C30)	27.03± 0.51	25.66 ± 2.18	14.86± 0.90	20.83± 0.70
Ciprofloxacin	29.63± 1.48	27.5± 1.57	16.3± 0.64	21.45± 1.59

-The value represents diameter of the zone of inhibition (mm) Mean ±Standard Error

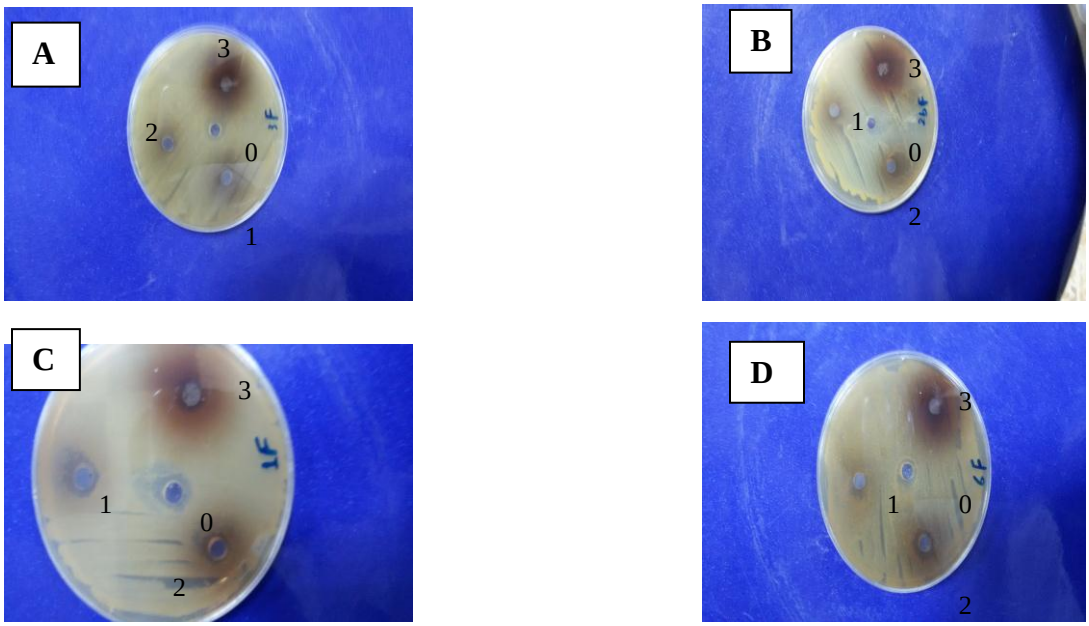

Figure (1): Effect of Pl. phenolic extract on tested pathogenic bacteria at different concentration: 0,1,2 and 3 (negative control, 5, 10 and 20 mg/ml). A: effects on the *B. subtilis*, B: effects on the *S. aureus*, C: effects on the *P. aeroginosa* , and D: effect on the *K. pneumonia*.

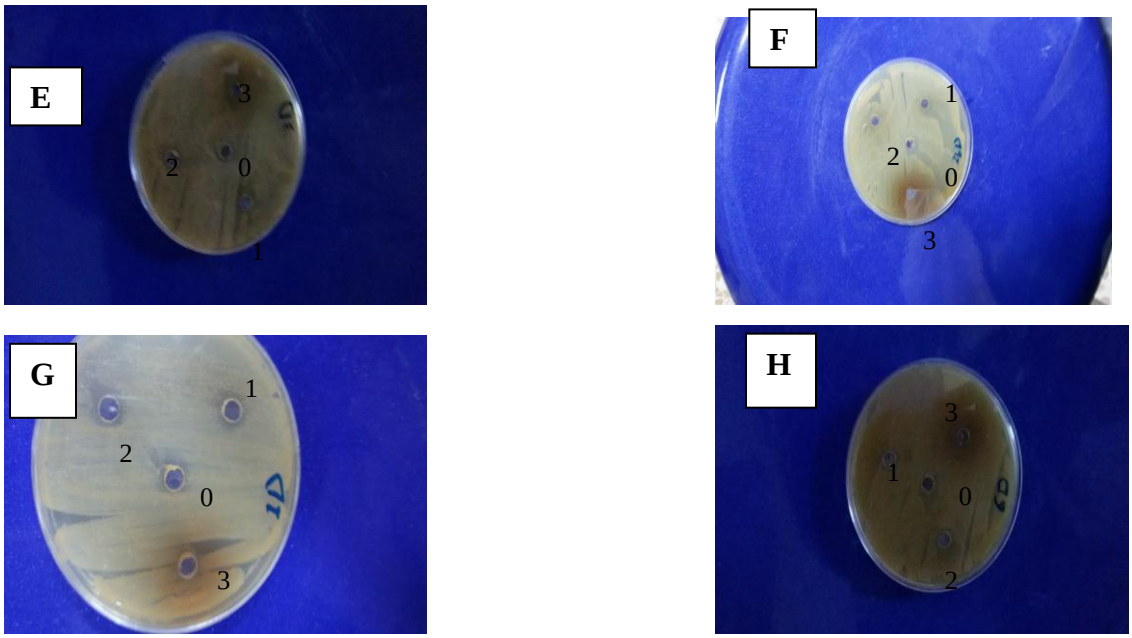

Figure (2): Effect of Ca. phenolic extract on tested pathogenic bacteria at different concentration: 0,1,2 and 3 (negative control, 5, 10 and 20 mg/ml). E: effects on the *B. subtilis*, F: effects on the *S. aureus*, G: effect on the *P. aeroginosa* , and H: effects on the *K. pneumonia*.

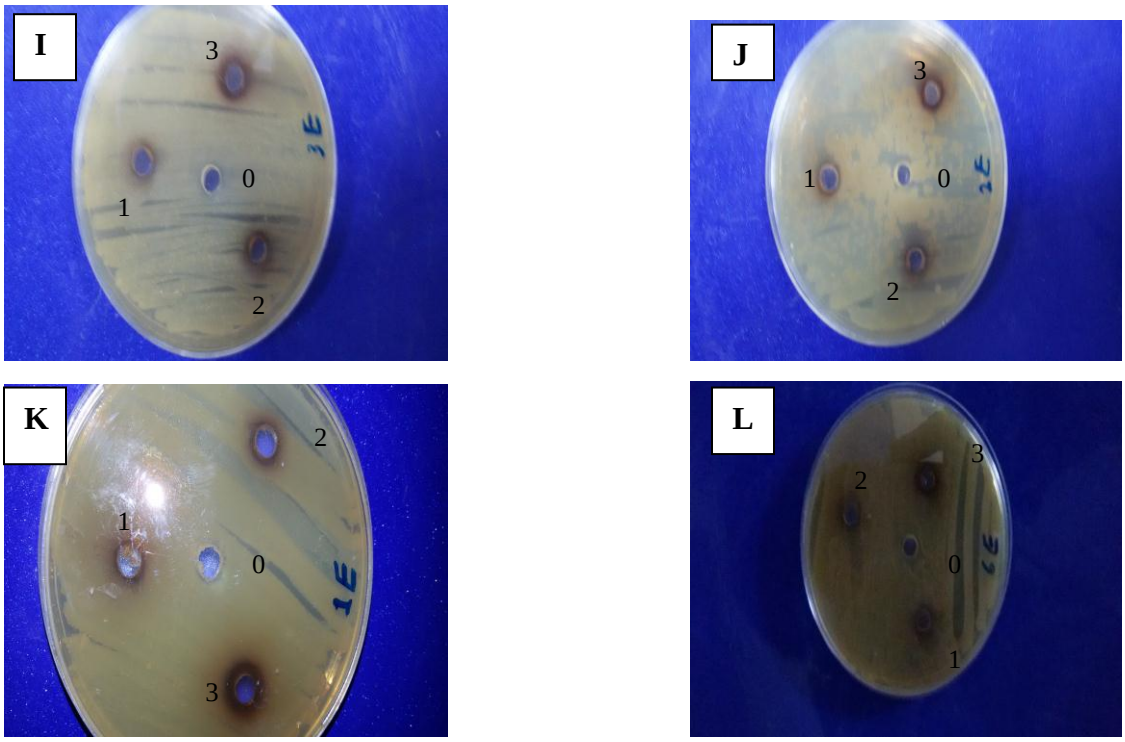

Figure (3): Effect of Eg. phenolic extract on tested pathogenic bacteria at different concentration:0,1,2 and 3 (negative control, 5, 10 and 20 mg/ml). I:effects on the *B. subtilis*, J: effects on the *S. aureus*, K: effects on the *P. aeroginosa* , and L: effect on the *K. pneumonia*.

Discussion

The present study revealed antibacterial activity of the phenolic extract of Pl., Ca. and Eg. against *B. subtilis*, *S. aureus*, *P. aeroginosa* and *K. pneumonia*. Our results agree with Mathew *et al*., (15), that he reported the antibacterial activity of methanolic extract of lanceolatae against both gram positive and gram negative bacteria. Also agree with Ehsan *et al* ., (16), that showed the antibacterial activity of aqueous leaf extract of lanceolata against pathogenic bacteria, and agreed with Pehlivan *et al*., (17) report that water extracts of Pl. produced the best antimicrobial effects. Among tested bacteria, B. subtilis was the most sensitive, while K. pneumonia moderately sensitive to phenolic extracts, this may be due to the cell walls of the gram negative bacteria less

permeable to antimicrobial compounds (15), or may be due to the presence of some active compounds in the extracts, which may inhibit or interfere with the bacterial growth (18, 19).Although, Packia lincy *et al*., (6) recorded that Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens, certain plant phenols can be effective inhibitors of chemical mutagens, in vitro, and /or carcinogensis in vivo. Ozan et al., (20) observed that phenolic substances found in plant extracts most likely act on the microbial membrane, or the surface of the cell wall, causing structural and functional damage.

Our results showed that Pl. recorded more effects against bacteria, that may be due to the presence of active compounds such as a spindinol, alkaloids, phenolics, saponins, tannins and flavonoids, that are known to have good antioxidant and antibacterial activity with high concentration more than the other plants (Ca. and Eg.), which had previously been reported from this plant (16, 15, 21). Phytochemical studies have also shown that genus plantago contain a great amount of phenlic compounds (flavonoids and tannins) (22).Also Eg. Reported the moderate activity against ehe bacterial growth that may be due to that Eg. Possess consistently the lowest values of total phenolic contents. While, our results disagree with Hassine *et al*., (23), that reported that essential oil from the flower of Ca. did not exhibit significant antibacterial activity, this is probably due to the different in the materials and methods and uses essential oil from the flower while in the present study used leaf phenolic extract which containing multiple organic components.

Conclusion

The present study showed the antibacterial activity of phenolic extracts of Pl. , Ca. , and Eg. And reported that Pl. more active than other plants. So this study support the traditional usage of this plant as antibacterial for the treatment pathogenic bacteria.

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