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Screening of Antibacterial Properties for Some Iraqi Plants Against *Salmonella typhimurium*

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Summary

This study was include the investigation of the antibacterial activity of ethanolic extract for (12) type of Iraqi plant leaves (*Allium porrum L, Apium graveolens Mill., Cassia angustifollia Vahl., Lawsonia inermis L., Lepidium sativum L., Malva sylvestris L., Monthapiperita L., Ocimmum bassillicum L., Pettroselinum crispum Mill., Raphanus sativus L., Sponacia oleraceal. and Trigonella foenum L.*). In different concentrations (20, 40, 60, 80, 100) mg/ml against *Salmonella typhimurium in vitro* using agar well diffusion method. All ethanolic extract of leaves showed a high activity against this bacterium except of *Cassia angustifollia Vahl*. as compared with the other extracts. The ethanolic extract of leaves of *Petroselinum crispum Mill* was showed the best activity with inhibition zone of twenty three mm in the concentration (100) mg/ml. This results confirm the antibacterial activity of these plants, and leaf components which are responsible for this action have to be isolated for using as therapeutic agents.

Conclusion: The present investigation together with previous studies provide support to the anti-*Salmonella typhimurium* properties of studied plant leaves. It can used as antibacterial supplement towards the development of the new chemotherapy . Additional *in vivo* studies and clinical trails would be needed to justify and further evaluate the potential of these plants as anti-*Salmonella typhimurium* agent in treatment of infectious diseases caused by this bacteria.

Keywords: Antibacterial activity, Iraqi plant leaves, *Salmonella typhimurium*
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فحص الصفات الضد بكتيرية لعدد من النباتات العراقية ضد جرثومة *Solmonella typhimurium*

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الخلاصة

تضمنت هذه الدراسة تقصي الفعالية الضد بكتيرية للمستخلص الاثيلي لـ (12) نوع من اوراق النباتات العراقية (*Allium porvum L., Apium graveolens Mill., Cassia angustifollia Vahl., Lawsonia inermis L., Lepidium sativum L., Malva sylvestris L., Monthapiperita L., Ocimmum bassillicum L., Pettroselinum crispum Mill., Raphanus sativus L., Sponacia oleracea L., and Trigonella foenum L.* . وبتراكيز مختلفة (20 ، 40 ، 60 ، 80 ، 100) ملغم/ملليتر ضد بكتريا *Salmonella typhimurium* خارج الجسم الحي باستخدام طريقة الانتشار بالاكار . اظهرت كل المستخلصات الاثيلية للاوراق فعالية عالية ضد هذه البكتريا ما عدا نبات السنامكي *Cassia angustifollia Vahl.* مقارنة مع المستخلصات الأخرى، اظهر المستخلص الاثيلي لاوراق نباتات المعدنوس *Petroselinum crispum Mill.* أفضل فعالية بمنطقية تثبيط (23) ملليتر في التركيز (100) ملغم/ ملليتر . تؤكد هذه النتائج الفعالية الضد بكتيرية لهذه النباتات ويمكن عزل مكونات الاوراق المسؤولة عن هذا التأثير لاستخدامها كعوامل علاجية .

الاستنتاجات , الدراسة الحالية مع الدراسات السابقة تقدم دعم للخصائص الضد بكتيرية لاوراق النباتات المستخدمة في هذه الدراسة ضد بكتريا *Salmonella typhimurium* ، ويمكن استخدامها كركيزة في تطوير عوامل علاجية جديدة.

يمكن القيام بدراسات سريرية داخل الجسم الحي لهذه النباتات لتوضيح وتقييم قوة هذه النباتات كعوامل ضد بكتيرية في علاج الاخماج المسببة من قبل هذه البكتريا.

Introduction

According to the reports of many researches, antibacterial resistance is a worldwide growing-problem (1). The problem of microbial resistance is growing and the outlook for the antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce the problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient (2, 3 and 4).

Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as pharmaceuticals antibiotics. Species of higher plants are much less surveyed for antibacterial activity (2 and 5). The use of herbs as complementary in the last 20-25 years, and according to world health organization (WHO) traditional medicines are relied upon by 65-80% of the words population

of their primary care needs (6). Antibacterial active principles isolated from higher plants is appears to be one of the important alternative approaches to contain antibiotic resistance and the management of disease. It is believed that plants based drugs cause less or no side effect when compared with synthetic antibiotics (2 and 7). *Salmonella typhimurium* is a pathogenic Gram-negative bacteria predominately found in the intestinal lumen (8). This bacterium is a serious public health problem in developing countries and represents a constant concern for the food industry (9). It causes enteric fever and invade intestinal mucosa and multiply in macrophages in intestinal lymph follicles, enter mesenteric lymph gland to blood and dissemination. The clinical features of infection include insidious onset malaise, anorexia maligns and headache (10 and 11). Shan *et al* examined 46 medicinal plant extract by agar well diffusion method against 5 foodborn bacteria and found that Gram-positive bacteria were generally more sensitive to the extract from Gram-negative bacteria(12).

In the present research, inhibitory effects of 12 Iraqi green leaves were investigated against *Salmonella typhimurium*.

Materials and methods

Plant material: Fresh leaf material of 12 species of plants were collected from Baghdad city, Iraq. Table (1). The leaves were washed thoroughly several times with running tap water and once with sterile distilled water. The leaf material was air-dried on a sterile blotter under shade (7). A voucher specimen of all plants has been deposited in the Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Iraq.

Preparation of extract: The leaves were extracted by dissolving in ethanol-distilled water and then collecting the supernatants. This process repeated three times. Finally the ethanolic extracts were incubated at room temperature for 48 hours. After dryness al extracts were dissolved in 5 ml of DMSO (dimethyl sulfoxide solvent) to get the concentration 20, 40, 60, 80, 100mg/ml. (13).

Preparation of bacterial inoculums: Bacterial suspension of *Salmonella typhimurium* was prepared according to McFarland nephdometer standard. A 24 hour old culture was used for preparation of bacterial suspension. The isolated colony was made in a sterile isotonic solution of sodium chloride (0.9%) and the turbidity was adjusted such that it contained approximately 1.5×10^8 cells/ml. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05 of 1.175% of barium chloride and 9.95ml of 1% sulphuric acid (14).

Antibacterial activity: The antibacterial assay was performed by the agar well diffusion method. The medium (Muller Hinton) was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121 C^o for 15 minutes. The Petriplates

were sterilized in hot air oven at 160 C^o for 1.5 hours. 30ml of sterile Muller Hinton agar was inoculated with microorganism (about 0.4ml according to McFarland standard). Pores were made on the medium by using a sterile borer and 0.1 ml of each extract was added to the respective pore. The Petriplates inoculated with micro-organism, containing extracts were kept in refrigerator at 4 C^o for 1 hour to facilitate the diffusion of extracts into the medium. After diffusion, the Petriplates were incubated at 37 C^o for 24 hours in an incubator and zone of inhibition was observed and measured using a scale (15).

Table (1) : List of species of tested plants for antibacterial activity [2].

Name of the plant	Family
Allium porrum L.	***Alliaceae***
Apium graveolens Mill.	***Apiaceae***
Cassia angustifollia Vahl.	***Leguminosae***
Lawsoing inermis L.	***Lythraceae***
Lepidium sativum L.	***Crucifera***
Malva sylvestris L.	***Malvaceae***
Mentha piperita L.	***Lamiaceae***
Ocimmum bassillicum L.	***Lamiaceae***
Petroselinum bassillicum L.	***Umbelliferae***
Raphanus sativus L.	***Brassicaceae***
Spanacia aleracea L.	***Chenopodiaceae***
Trigonella foenum L.	***Papilionaceae***

Results and Discussion

Recently, much attention has been directed towards plant extracts and biological active compounds isolated from popular plant species (16). The use of medicinal plants plays a vital role in developing countries and these plants may offer a new source of antibacterial agents like flavorides, phenolic, polyphenols, tannins, terpenoides and sequitorpenes with significant activity against infective microorganisms (17, 18 and 19) . The anti-*Salmonella typhimurium* activity of ethanolic extracts for 12 type of Iraqi plant leaves are presented in Fig. (1 and 2) and table 2.

The results shown that all the ethanolic extracts (except one) prouduced zones of inhibition in the agar well diffusion method. We found that *Peterselinum crispum* was the most active tested plant against *Salmonella typhimurium* with inhibition zone 23 mm in the concentration 100mg/ml, followed by *Trigonella foenum L.* and *Malva sylvestris L.*with inhibition zones 21, 20 mm in the same concentration. The antibacterial effect was demonstrated by previous workers (12, 16, 20 and 21). The other plants like *Allium porrum, Apium graveolens, Lawsonia inermis L., Menthe piperita, Ocimum basillicum, , Lepidium sativum Raphanus sativus, Spanacia aleracea,* have a good *anti-Salmonella typhimurium* activity in all concentration. To some extent, these results were similar to

those of previous studies (12, 22, 23, 24, 25 and 26). The organic solvent extract exhibit greater antibacterial activity because the antibacterial principles were either polar or not polar and they were extracted only through organic solvent medium. Organic solvent extraction was suitable to verify the antibacterial properties of medicinal plants and they supported by many investigators (27, 28 and 29). In the other hand the ethanolic extract of *Cassia angustifollia* leaves was the least effect as compared with the other extracts. This suggests may be some particular anti-*Salmonella* substances in these extracts, which are lack in case *Cassia angustifollia* plant (12). The method of extraction (the ethanolic extract possess antibacterial activity than methanolic extract), the plant material and it's form (water crushed or finely powdered) affected their antibacterial activity, also the filtration of extract which might have led to remove of the antibacterial activity components (3, 30 and 31). The obtained results may provide a support to use these plants in traditional medicine.

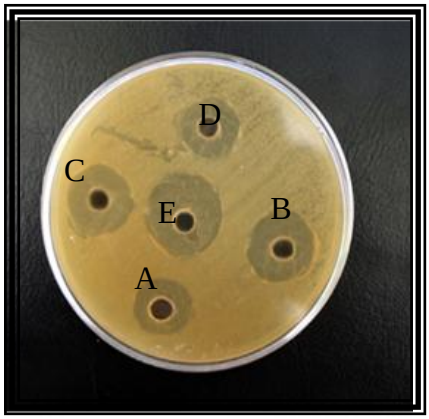

Fig.(1):The antibacterial activity of *Petroselinum crispum* leaves against *Salmonella typhimurium*
A : 20 mg/ml of ethanolic green leaves extracts
B : 40 mg/ml of ethanolic green leaves extracts
C : 60 mg/ml of ethanolic green leaves extracts
D : 80 mg/ml of ethanolic green leaves extracts
E : 100 mg/ml of ethanolic green leaves extracts

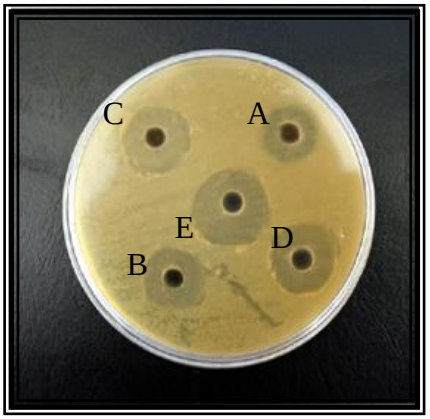

Fig. (2) : The antibacterial activity of *Trigonella foenum* leaves against *Salmonella typhimurium*

Table 2 : Results of Screening of 12 ethanolic green leaves extracts against *Salmonella typhimurium*

Name of plants	Concentration (mg/ml)	Zone of inhibition(mm)
Petroselinum crispum Mill.	20	17
	40	18
	60	18
	80	21
	100	23
Trigonella foenum L.	20	7
	40	12
	60	15
	80	19
	100	21
Malva sylvestris L.	20	6
	40	8
	60	10
	80	17
	100	20
Allium porrum L.	20	12
	40	13
	60	15
	80	15
	100	18
Apium graveolens Mill.	20	-
	40	6
	60	10
	80	13
	100	18
Lawsania inermis L.	20	7
	40	7
	60	9
	80	13
	100	17
Menthe pipertita L.	20	6
	40	8
	60	11
	80	14
	100	15
Ocimmum bassillicum L.	20	8
	40	10
	60	11
	80	14
	100	14
Lepidium sativum L.	20	-
	40	8
	60	8
	80	11
	100	12
Rophanus sativus L.	20	7
	40	8
	60	10
	80	11
	100	11
Spanacia oleracea L.	20	6
	40	8
	60	8
	80	9
	100	11
Cassia angustifollia Vahi.	20	-
	40	-
	60	-
	80	
	100	4

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