

Research Article

The Inhibition Potential of Oregano (*Origanum Vulgare*) Extract Against *Citrobacter freundii* Invitro and Invivo

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Abstract

The study was designed to demonstrate the antimicrobial and antioxidant effects of *Oregano* against *C. freundii*. The study consist of two parts, the first part (invitro) including study the sensitivity of *C. freundii* against safflower extract and antibiotics. where, the results showed very high inhibition efficacy of extract, which reached to 30 mm, while Neomycin inhibition zone reach to 14mm, Novobiocin inhibition zone reach to 9mm and Tetracycline inhibition zone reach to 9mm. In the second part (in vivo) used 24 albino rats and divide to six groups (each group consist 4 rats). control group, group injected with (1×10^5 CFU/ml/25mg) bacteria, group injected with (1×10^5 CFU/ml/25mg) bacteria and treated with neomycin, group injected with (1×10^5 CFU/ml/25mg) bacteria and treated with novobiocin, group injected with (1×10^5 CFU/ml/25mg) bacteria and treated with tetramycine, group injected with (1×10^5 CFU/ml/25mg) bacteria with extract. The MDA levels in infected group and treated groups with antibiotics increased and GSH levels in infected group and treated groups with antibiotics show decreased ($P < 0.05$) compared control rats. In treated group with extract, MDA and GSH levels show on-significant change compare with control rats. It was concluded from present study that the *Oregano* has an antimicrobial and antioxidant activity against *C. freundii*.

Keywords: *C. freundii*; *Oregano*; oxidative stress; MDA; Glutathione.

الخلاصة

اجريت الدراسة الحالية لظهور التأثيرات كمضاد بكتيري ومضاد اكسدة لمستخلص نبات المردقوش ضد *C. freundii*. تكونت الدراسة الحالية من جزئين: الجزء الاول (خارج الجسم) والذي تضمن حساسية *C. freundii* ضد المستخلص والمضادات. حيث اظهرت النتائج فعالية تثبيطية عالية وصلت الى تكوين منطقة تثبيط قطرها 30mm, منطقة تثبيط مضاد النيوميسين وصلت 14mm, منطقة تثبيط مضاد النوفوبايوسين وصلت 9mm و منطقة تثبيط مضاد التتراسايكلين وصلت 9mm. ام الجزء الثاني من الدراسة (داخل الجسم) تم استخدام 24 الجرذان البيض ووزعت الى ست مجاميع (كل مجموعة تحتوي 4 جرذان), مجموعة السيطرة, المجموعة الثانية والتي حققت بجرعة (1×10^5 CFU/ ml/25mg) من البكتيريا, المجموعة التي حققت بجرعة (1×10^5 CFU/ ml/25mg) من البكتيريا وتم معالجتها بمضاد النيوميسين, المجموعة التي حققت بجرعة (1×10^5 CFU/ ml/25mg) من البكتيريا وتم معالجتها بمضاد النوفوبايوسين, المجموعة التي حققت بجرعة (1×10^5 CFU/ ml/25mg) من البكتيريا وتم معالجتها بمضاد التتراسايكلين, المجموعة التي حققت داخل الصفاق بجرعة (1×10^5 CFU/ ml/25mg) من البكتيريا وتم معالجتها بالمستخلص النباتي. التشخيص لمضادات الاكسدة اظهر زيادة معنوية في مستويات MDA وانخفاض معنوي في مستويات الكلوتاثيون في المجاميع المخمجة والمجاميع المعالجة بالمضادات الحيوية. بينما المجاميع المخمجة بالبكتيريا والمعالجة بالمستخلص النباتي فلم يكن هناك اي تغيرات معنوية في مستويات MDA والكلوتاثيون مقارنة مع مجموعة جرذان السيطرة. استنتج من هذه الدراسة بان مستخلص نبات المردقوش فعالية مضاد اكسدة ومضاد بكتيري ضد بكتيريا *C. freundii*.

Introduction

Citrobacter, Gram negative bacilli belonging to the family Enterobacteriaceae, species are considered to be environmental contaminants or harmless inhabitants in intestinal tracts of man and animals [1]. Rods haven't spores back

to the family Enterobacteriaceae and, usually utilize citrate as a sole carbon source. These facultative anaerobes typically are using peritrichous flagella to motility [2]. These bacilli are commonly distributed in soil, sewage, water and food. The importance of this species lies in their association with serious

nosocomial infections and high degree resistance to common antimicrobial agents used for the treatment of various infections [1] [2] [3]. *Citrobacter* (*C. freundii*, *C. diversus*, and *C. amalonaticus*) have been linked to a number of diseases, including those of the urinary tract, respiratory tract, wounds, bones, peritoneum endocardium, meninges, intestines and blood stream. Some serotypes of *C. koseri* (*diversus*) can also be enteropathogenic causing diarrhea [4] [5] [6] [7] [8] [9].

Oregano (*Origanum vulgare* L.) is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia [10]. Oregano has been antimicrobial [11], antifungal [10], insecticidal [12] and antioxidant properties [13]. The aim of study is detect the activity of *Origanum vulgare* extract against *C. freundii*.

Materials and Methodology

Plant extract

Origanum vulgare Linn leaf were purchased from the local market of Baghdad. Ethanolic extract of plant was weighting 20 of powder and dissolved in 500 ml of 70% ethanolic alcohol extract in Erlenmeyer flask and freezing in deep freeze for 9 days then on magnetic stirrer for 2 hours at room temperature. The sediments filtered and then the mixture was filtered by using filter paper. The supernatant was evaporated to dryness (45°C) under reduced pressure in a rotary evaporator. The crude extract then was kept at -20°C until the time of use [14].

Citrobacter freundii

Different clinical samples such as urine, diarrhea specimens, sputum and pus collected from children suspected of bacterial infection.

Cultivation and Identification

The collected samples were inoculated on MacConkey agar. Then, all plates were inoculated at 37 °C. The motility test for bacteria was done. Isolated and identified of *Citrobacter freundii* colonies were done according to MacFaddin (2000), they method to isolation include; positive for H₂S, urease, catalase, citrate, lactose fermentation, MR and negative for oxidase, VP and indole. *C.*

freundii was identified by maltose fermentation and ornithine decarboxylase production [15].

The effectiveness inhibitory

The inhibition potential of antimicrobial agents (Neomycin (30 µg) Novobiocin (30 µg) Tetracycline (10 µg), Gentamycin (10 µg) and leaf extract against *C. freundii* was done by using disk diffusion method [16]. The Muller-Hinton agar was evenly inoculated with *C. freundii*. The antibiotics (Gentamycin, Neomycin, Novobiocin and Tetracycline) and safflower extract discs were applied to the surface of agar and incubated overnight at 37 °C. The diameter of growth zone has been measured.

Animal model

24 adult male rat, (wt 250-290 g/ age: 6-10 mon) obtained from requirements medicals company – Samara, and kept with standard diet.

Experimental design

24 adult male rats were using and divided to six groups (each group consist 4 rats) as follow:

1. Control group.
2. Rats injected (intravenously) with 1×10^5 CFU/ ml/25mg [17], and killed after the infection happen.
3. Rats injected (intravenously) with 1×10^5 CFU/ ml/25mg bacteria. Then, treated (orally) with Neomycin (10mg/kg) for three week.
4. Rats injected (intravenously) with 1×10^5 CFU/ ml/25mg bacteria. Then, treated (orally) with Novobiocin (10mg/kg) for three week.
5. Rats injected (intravenously) with 1×10^5 CFU/ ml/25mg bacteria. Then, treated (orally) with Tetracycline (10mg/kg) for three week.
6. Rats injected (intravenously) with 1×10^5 CFU/ ml/25mg bacteria. Then, treated (orally) with 1ml/100mg/kg extract for three week.

Prepare of blood solution

Blood collected, under anesthesia, by cardiac puncture and put in test tubes. The tubes were

centrifuged 5000 cycle/min for 10 min. then; the serum was taken and stored until used. Measurement of serum MDA was based reaction with thiobarbituric acid and GSH was measured by CDNB and buffer [18].

Statistical analysis

The data was analyzed by using a Minitab program. The program using Analysis of Variance (ANOVA) test, in order to evaluate the significance of variability between experimental groups.

Results and Discussion

Inhibition potential of extract and antibiotic toward bacteria

The inhibition potential results of extract against bacteria showed very high inhibition efficacy of plant extract compare with antibiotic as shown in Figure 1 and Table 1

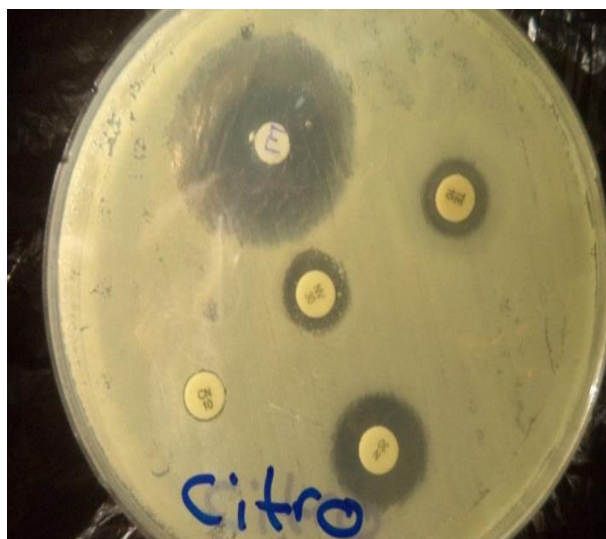


Figure 1: inhibitory potential of plant extract and antibiotics against bacteria inhibitory potential of plant extract and antibiotics against bacteria. E: extract, N: neomycin, NV: novobiocin, TE: tetracycline and CN: Gentamycin

Antioxidant factors (MDA, GSH, catalase and TAC)

The MDA levels in both infected group and treated groups with antibiotics showed significant increased ($P < 0.05$) compared with control rats. Group which treated with leaf

extract show non-significant change compare with control rats. GSH levels in infected group and treated groups with antibiotics showed a significant decreased ($P < 0.05$) compared control rats. As shown in Table 2.

Table 1: Inhibitory potential of plant extract and antibiotics against bacteria.

Treatments	Inhibition zone (mm)
Plant extract	30
Neomycin	14
Tetracycline	9
Novobiocin	9
Gentamycin	0

Table 2: inhibitory potential of plant extract and antibiotics against bacteria.

Parameters Groups	MDA (mmol/l)	GSH (mol/l)
Control group	1.64 ± 0.15 c	0.84 ± 0.1 a
Infected group	2.03 ± 0.13 b	0.52 ± 0.04 b
Infected group + Neomycin	2.3 ± 0.2 a	0.39 ± 0.02 c
Infected group + Novobiocin	2.38 ± 0.95 a	0.37 ± 0.021 c
Infected group + Tetracycline	2.4 ± 0.12 a	0.39 ± 0.08 c
Infected group + Extract	1.7 ± 0.09 c	0.81 ± 0.06 a

The results showed high inhibition potential of ethanol extract of plant leaf and flower against bacteria compare with antibiotics. In a study carried by Dorman and Deans (2000) referred that Oregano has antibacterial properties, they found that the inhibition zone caused by Oregano against *C. freundii* reached to 29.2 mm [19]. In another study of Masood *et al.* (2007) referred that Oregano has antibacterial activity. They found that inhibition zone reached to 24 mm against *C. freundii*. Suggested that these activities because of the different prosperities they have reported by antifungal, anticoccidial, antispazmolytic, antibacterial and antioxidant [20]. The components of oregano are gamma-cariophyllene, rho-cymenene, Alpha-pinene, canfor, carvacol and thymol [21]. Among them thymol and carvacrol are the main components of the essential oil of oregano [22], which are responsible for its antioxidative, antimicrobial and antifungal effects [23]. Darogha (2009)

showed that there isn't inhibition zone of gentamycin and different types of antibiotics (Ceftazidime, Amoxillin, Ciprofloxacin, Cefotaxime and Vancomycin) against *C. freundii* [24].

The results of the present study referred that the oregano has an antioxidant properties. Where, after elevated the MDA levels and decreased the GSH levels in rats by bacteria, the oregano extract lead to MDA and GSH levels back to normal ranges. Omer *et al.* (2013) referred that oregano has been antioxidant properties; they found that the MDA increased and GSH decreased in rabbits that fed by high fat diet, but after using oregano extract in treatment, they found that MDA and GSH levels back to normal ranges [25].

Conclusions

The present study was designed to show the role of *Oregano* against *C. freundii*. It was concluded that the Oregano has been antimicrobial activity against *C. freundii* according to the in vivo (sensitivity test) and in vitro (by using mice) experiments.

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