التأثير الضد بكتيري لنبات الحنة باستعمال ثلاثة انواع من مستخلص ورق الحنة خارج الجسم

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الخلاصة

تضمنت هذه الدراسة تقصي التأثير الضد بكتيري لثلاثة انواع من مستخلص ورق الحنة (الماء ، والميثانول ، والكلوروفورم) ، وبتراكيز مختلفة (40، 80 ،120) ملغم/مل ضد اربعة انواع من سلالات البكتريا *Staphyllococus aureus, Bacillus subtilis, Pseudomonos aerogenosa and Eschorichia coli* في الخارج باستخدام طريقة الانتشار بالاكار .

اظهر مستخلص الماء اعلى فعالية ضد البكتريا ثم يليه مستخلص الميثانول ، بينما اظهر مستخلص الكلوروفورم اوطأ فعالية . لوحظ اعلى منطقة تثبيط لمستخلص الماء ضد بكتريا *seudomonos aerogenosa* (25) مليمتر ، وبتركيز (120ملغم/مل) بينما اقل منطقة تثبيط لبكتريا *Bacillus subtilis* (9مليمتر) وللتركيز نفسه .

النمو البكتيري تم تثبيطه بدرجات مختلفة مع زيادة تركيز المستخلص ، وهذه النتائج تؤكد الفعالية الضد بكتيرية لاوراق الحنة وتدعم الاستعمال التجاري لهذا النبات بوصفه علاجاً للاخماج البكتيرية.

الكلمات المفتاحية : اوراق الحنة ، *Lawsonia inerims , Staphyllococus aureus, Bacillus subtilis, Pseudomonos aerogenosa, Eschorichia coli* ، خارج الجسم

Antibacterial Effect of *Lawsonia inerims linn.* (*Henna*) Using Three Types of Leaf Extract *In Vitro.*

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Abstract

This study was done to investigate the antibacterial effect of the three types of *Lawsonia inermis linn (henna*) leaf extracts (water, methanol and chloroform) in different concentrations (40, 80, 120) mg/ml against four strains of bacteria (*Staphyllococus aureus, Bacillus subtilis, Pseudomonos aerogenosa and Eschorichia coli*) *in vitro* using ager well diffusion method.

Water extract showed the highest antibacterial activity, followed by methanol extract, while the chloroform extract showed the lowest activity. The maximum inhibition zone of water extract was observed against *, Pseudomonos aerogenosa* (25mm) in the concentration (120) mg/ml, while the minimum zone of inhibition (9mm) was in *Bacillus subtilis* in the same concentration.

The growth of all bacteria was inhibited by varying degrees with the increase of the extract's concentrations. These results confirm the antibacterial activity of *henna* leaves and support the traditional use of the plant in therapy of bacterial infections.

Keywords: *Henna* leaves , *Lawsonia inerims*, *Staphyllococus aureus, Bacillus subtilis, Pseudomonos aerogenosa, Eschorichia coli, in vitro*

Introduction

Some bacteria are extremely pathogenic causing serious human infection . The discovery of antibiotics to combat these pathogens marked a resolution in the 20th century[1]. Unfortunately, because of the inappropriate use of antibiotics in human and veterinary medicine, certain strains of bacteria developed the ability to produce substances which block the action of antibiotics or change their target or ability to penetrate cell [2]. Therefore, disease caused by microbes that become resistant to antibiotic drug therapy are an increasing public health problem. Tuberculin, gonorrhea, malaria and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. However, a large part of the problem is due to our increasing use and miss use of existing antibiotics in human and veterinary medicine and in agriculture [3] .To substitute synthetic antibiotics, many of today modern and effective drugs have their origin in traditional folk medicine [4] . Plants have been used to treat human , animals and plant diseases, from time immemorial .Also herbal medicines have been known to man for centuries[5]. Therapeutic efficacy of many indigenous plants for many disorders had been described by practitioners of traditional medicine[6,7].

The henna plant *lawsonia inermis linn*, is one of such plant known since healing attributes and is now the subject of intense scientific study [8,9] . The plant belong to the family lythraceae and in Iraq is traditionally used to develop a red or black coloring to hands , feet and hairs in some occasions such as weddings and religious festivals. It is a perennial shrub branching profusely and reaching a height of up to six meters. The leaves of the plant are small , lanceolate , dark – green and glabrous , opposite , with very short petioles .Flowers are small , white or pale pink colored and fragrant . The plant leaf contains a red orange color

component lawsone (2 – hydroxyl – 1,4 – Napthoquinone). The capsulated fruits are brone in clusters, green in color.

Henna is believed to have a cooling effect on the body and in the past it was used to bring down fever. As amedicinal plant, because of its attributed antibacterial, antifungal, antiamoebiosis, astringent, antihemorrhagic, hypotensive and sedative effects[10] .

According to phytochemical analysis of *henna* , powdered leaves contain about 0.5 – 1.5 % lawsone , the chief constituent responsile for the dyeing properties of the plant . *Henna* also contains mannite , tannic acid , mucilage ,gallic acid and napthoquinone[11,12] . Antimicrobial properties of lawsonia inermis were investigated by several workers from around the world[13,14.15].

In Iraq, the antibacterial effect of *henna* plant was studied by AL-Rubiay *et al* in Basrah city[16].

The present study was planned to find out the antibacterial activities of *henna* leaves and their efficacy against different bacterial strains.

Materials and Methods

- **Plant material**

Leaves of *lawsonia inermis linn*. (*Henn*) were purchased from one of the lawns in Baghdad city. Fresh leaves were dried in shade , then grounded to powder .

- **Preparation of extracts**

Coarsely powdered plant material were successively soxhlet extracted with CHCL, and MeOH for 24 hrs. The collected extracts were concentrated by evaporation, and the residues were separately dissolved in the same extracting solvent of the concentrations (40, 80, 120)mg/ml and kept in refrigerator till use. In addition ,water extracts were prepared by adding distilled water to coarsely powdered plant material in a conical flask and left to soak overnight. The residue was then filtered and the solution was used immediately [3, 17] .

- **Bacterial strains**

- micro-organisms used

Gram-positive bacteria:*Staphyllococcus aureus,Bacillus subtilis.*

Gram-negative bacteria:*Pseudomonas aeruginosa,Escherichia coli.*

All isolates were obtained from Health Central Laboratory, Baghdad, Iraq. The bacteria was cultured on nutrient broth at $37C^{o}$ for 24 hr.

- Preparation of inoculum

Suspension of organism was prepared as per Mcfarland nephalometer standard. A 24 hours old culture was used for the preparation of bacterial suspensions.The suspension of organism was made in a sterile isotonic solution of sodium chloride(0.9%w/v) and the turbidity was adjusted such that it contianed approximately $1.5x10^{8}$cells/ml [18].

- **Agar well diffusion method**

The medium (Muller Hinton Agar) was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121˚c for 15 minutes.The Petri plates were washed thoroughly and sterilized in hot air oven at 160˚c for (1.5) hour . Thirty ml of sterile nutrient agar was seeded by organsims(about 0.2ml according to Mcfarlands standard). Pores were made on the medium using asterile cork borer and 0.1ml of each extract (water, methanol and chloroform) was added separately to respective pore. The Petri plates seeded with organisms contianing extracts were kept in refrigerator at 4˚c for 1hour to facilitate the diffusion of the extracts into the media. After diffusion the Petri plates were incubated at 37˚c for 24 hours in an incubator and zone of inhibition was observed and measured using a scale.

Results and Discussion

The results of the antibacterial activity of leaves of *lawsonia inermis linn* are tabulated in Table(1). As a general rule, plant is considered active against bacteria when the zone of inhibition is greater than 6 mm [3, 19]. Results presented in table (1) indicated that all of the (3) tested leaf extracts of *henna* plant (*lawsonia inermis Linn.*) at different concentrations suppressed the growth of the tested pathogenic bacteria at varying degrees. Antibacterial activity may be due to numerous free hydroxyl that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them in active [16, 20].

Results obtained from *in vitro* antibacterial activity showed that water extract was superior in suppressing the bacterial growth, followed by methanol and chloroform extract respectively also the antibacterial activity was increased with the increase of the extract concentration. The maximum inhibition zone of water extract (in the concentration 120mg/ml) was observed against *Pseudomonas aerogenosca* (25mm) followed by *Staphyllococcus aureus* (20mm) and (14mm) for *Escherichia coli*, and a minimum zone of inhibition (9mm) in *Bacillus subtilis*.

The weakest activity of chloroform extract with a maximum zone of inhibition (16mm) was observed against *Pseudomonas aerogenosa* and a minimum zone of inhibition (7mm) in *Bacillus subtilis* in the same concentration of the extract (120mg/ml).

The present results are in close agreement with previous reports elsewhere using the same plant [3 ,17,19]. The antimicrobial substances in *henna* leaves are highly soluble in water, partially soluble in (70%) ethyl alcohol, and heat-stable [15]. The chloroform showed the weakest activity compared to other types of extract (water and methanol), and this may be due to the large quantity of active substances that were precipitated during the extraction process due to the effect of solvent itself [16, 20]. It was clear from this study that the solvent of extraction and method of extraction affected the degree of antibacterial activity .

The aqueous extract of henna plant had more effect against bacteria that investigated by many studies [3, 15, 19, 20].

Conclusions and Recommendations

It is not possible to make direct correlation between the observed activity of the plant extract *in vitro* and the actual effects when used *in vivo* for the disease observed by indigenous people and tradional haders.

The present study identifies Iraqi henna (*Lawsonia inermis L.*) as potensial source of biological antibacterial, since it showed a high activity against wide spectrum of bacteria which enable only human pathogenic bacteria to be killed without any side effects.

Additional deep research is necessary to isolate and characterize their active compounds for pharmacological testing.

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Table (1) *In Vitro* antibacterial activity of *lawsonia inermis Linn*. leaf extracts.

Tested isolated	Extract type	Concentration (mg\ml)	Zone of inhibition (mm)
Pseudoomonas aerugenosa	Water	40	20
		80	22
		120	25
	Methanol	40	16
		80	18
		120	19
	chloroform	40	14
		80	15
		120	16
Staphyllococcus aureus	Water	40	18
		80	19
		120	20
	methanol	40	15
		80	16
		120	17
	chloroform	40	12
		80	14
		120	15
Escherichia coli	Water	40	10
		80	12
		120	14
	methanol	40	8
		80	9
		120	10
	chloroform	40	0
		80	7
		120	8
Bacillus subtilis	water	40	7
		80	8
		120	9
	methanol	40	6
		80	7
		120	8
	chloroform	40	0
		80	6
		120	7