Inhibition of Bacterial Growth by *Lawsonia inermis* (henna) Leaf Extracts In Vitro

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Abstract

Leaf samples of *Lawsonia inermis* were collected from Basrah city, South of Iraq to examine their antimicrobial activity . The effects of water and chloroform crude extracts of the leaves in different concentrations were obtained and bioassayed *in vitro* for its bioactivity to inhibit the growth of six types of bacteria . The extract of water was clearly superior for all bacteria especially the bacteria *Staphlylococcus aureus* (inhibition zone was 21mm in concentration 70mg/ml) from gram positive bacteria, and *Klebsiella pneumoniae* (inhibition zone was 20mm in the same concentration) , and the growth of all bacteria was inhibited to varying degrees by increasing the concentration of the henna leaves and are commonly known to possess antimicrobial activity . These results confirm the antibacterial activity of henna leaves and support the traditional use of the plant in therapy of bacterial infections .

Introduction

The plant *Lawsonia inermis* (henna) belongs to the family Lythraceae and is best known for its colouring matter contained in the leaves [1] , and it is a glabrous much branched shrub or quite a small tree with grayish –brown bark [2] .

The henna plant is one such plant known since with healing attributes and is now the subject of intense scientific study [3,4] . The plant constituents are made up of mannite , tannic acid , mucilage and gallic acid , but the main constituent is 2-hydroxynaphthoquinone (lawsone) , known to be the major bioactive constituent , dried powdered leaves of henna contain about 0.5-1.5% lawsone . Henna is naturalized and cultivated in the tropics of America , Egypt , India and parts of the midlle east [2,5].

In early Islamic culture henna usage is very evident in the book Prophetic Medicine where the medicinal practices of Prophet Mohammed (PBUH) , as mentioned by his followers and others that were close to him in his household , were recorded [6]. The therapeutic efficacies of many indigenous plants for several disorders have been described by practitioners of traditional herbal medicines [7] . Being sources of many life sustaining metabolites , the research is still on for plants to be used in healing . This in part is due to the growing problem of worldwide antibacterial resistance . Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world [8,9] .

Indeed the synthesis and evalution of antimicrobial activity of the natural naphthoquinone products and their derivatives has been investigated [10,11] . Although naphthoquinones do appear to exhibit a wide spectrum of biological activities , the mechanism/s of action remains somewhat unclear[12] . However lawsone was shown to elicit in vivo lower toxic effects in mussel tissues than tissues in higher organisms . This may be due to the lower detectable levels of xanthine oxidase in the invertebrate mussels [13,14] .

Leaves of henna are useful to bring down the severity of many medical problems like dysentery , diseases of the spleen , lumbago , bronchitis and syphilitic eye infection [5] . Based on these observations this study was undertaken to determine the possible antimicrobial activities of *Lawsonia inermis* against different bacterial species .

Materials and Methods

Plant material : The henna plant *Lawsonia inermis* leaves samples used in this study were collected from Basrah city ,South of Iraq . Fresh leaves were dried in shade , then were ground to powder .

Preparation of extracts : Dried *Lawsonia inermis* leaves were powdered mechanically and mixed with sterile distilled water in a conical flask and left to soak overnight at room temperature , the residue was then filterated . The filterate was then mixed with the chloroform in a separating funnel , the mixture was shaken until separation was observed in form of two layers ; the water and the chloroform extract . The different layers were run out into separate beakers and placed in an oven to dry at 50c .Residues and the extracts were made into suspensions using sterile distilled water at concentrations (30 , 50 , 70) mg/ml .

Bacterial species : Three types of gram positive bacteria namely *Staphylococcus aureus , Bacillus subtilis* and *Streptococcus pyogenes* , and three types of gram negative bacteria namely *Escherichia coli , Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were used . The bacteria was cultered on nutrient broth (oxoid) at 37c for 24h .

Determination of diameter of growth inhibition : The agar well diffusion method was employed . Four 9mm wells were made in each plate of solidified nutrient agar . 0.5ml of each concentration of the extracts was measured out and mixed with 0.3g of agar . The well was carefully filled with this mixture and left to solidify. Each of the plates was seeded with each of the different organisms and incubated in the incubator for 24h . The measurements were then taken again by using linear measurement method [2,15] .

Results and Discussion

The plant leaves of *Lawsonia inermis* are commonly used in various dye industries for commercial purposes. Many studies showed that these leaves containes naphthoquinone (lawsone) in higher concentrations which was proved to have analgesic, anti inflammatory and antipyretic effects in rat models.

Inhibitory action of henna was shown against both gram positive and gram negative bacteria . Antibacterial activity is recorded when the zone of inhibition is greater than 6mm [2]. Results as shown in Table 1and 2 indicated that all of the two tested leaf extracts of henna plant (*Lawsonia inermis*) at different concentrations suppressed the growth of the tested bacteria at varying degrees . Extract of water was clearly superior in bioactivity as compared to that of chloroform . The maximum inhibition zone was found in 70mg\ml water extract concentration and it was 21mm in the bacterial isolate of *Staphylococcus aureus* , while the least inhibition zone in the same concentration was 14mm for the pathogen *Pseudomonas aeruginosa* . The activity increased with the increase of the extract concentration . Even in the lowest concentration of water extract i.e., 30mg\ml , all of the bacterial isolates showed substantial inhibition in their respective growth . The minimum inhibition zone in 30mg\ml concentration was observed in *Ps.aeruginosa* with 9mm inhibition zone Table (2). The chloroform extract of the plant showed maximum activity against the *Klebsiella pneumoniae* where the zone of inhibition was 12mm and the weakest activity was observed against *Streptococcus pyogenes* and *Pseudomonas aeruginosa* (8mm) in the same concentration (30mg\ml) .This data is in close agreement with previous reports elsewhere using the same plant [2,5] .

It is not possible to make a direct correlation between the observed activity of the plant extracts *in vitro* and the actual effects when used *in vivo* for the diseases observed by the indigenous people and traditional healers [16] . Therefore , it is important that the plant should also be further investigated to evaluate the significance of these extracts , clinical role

and the medical system of indigenous people . Additional deep research is necessary to isolate and characterize their active compounds for pharmacological testing.

Conclusions

It is clear that *Lawsonia inermis* leaves as an extract may be useful as an antimicrobial agent against the above mentioned bacteria . Further studies need to be undertaken regarding toxicity , safety and absorption pattern of the active ingredients . Isolation of the active ingredient from *Lawsonia inermis* will facilitate further studies . The present study identifies henna as source of biological antimicrobial, since it showed a high activity against wide spectrum of bacteria to be killed without any side effects and/or bacterial resistance as current synthetic antibiotics are doing and this specifity appears as additional point in the natural antibiotics research.

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Table (1)*: In vitro* antibacterial activity of *Lawsonia inermis* leaf extracts on gram positive bacteria

Organisms	Extract	Concentration mg/ml	Zone of inhibition mm,diameter
Staphylococcus aureus	water	30	11
		50	17
		70	21
	chloroform	30	9
		50	16
		70	19
Streptococcus pyogenes	water	30	11
		50	14
		70	17
	chloroform	30	8
		50	12
		70	15
Bacillus subtilis	water	30	11
		50	15
		70	18
	chloroform	30	10
		50	12
		70	17

Table(2):*In vitro* antibacterial activity of *Lawsonia inermis* leaf extracts on gram negative bacteria

Organisms	Extract	Concentration mg/ml	Zone of inhibition mm, diameter
Escherichia coli	water	30	13
		50	17
		70	19
	chloroform	30	11
		50	17
		70	18
Pseudomonas aeruginosa	water	30	9
		50	11
		70	14
	chloroform	30	8
		50	11
		70	14
Klebsiella pneumoniae	water	30	13
		50	18
		70	20
	chloroform	30	12
		50	17
		70	20

تثبيط النمو البكتيري باستعمال مستخلصات ورق نبات الحنة (*Lawsonia inermis*) خارج الحي

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الخلاصة

جمعت اوراق نبات الحنة *Lawsonia inermis* من مدينة البصرة لفحص نشاطها الضد مايكروبي . تم الاختبار الحيوي خارج الحي لمعرفة تأثير مستخلصات الماء والكلوروفورم للاوراق بتراكيز مختلفة وملاحظة تأثيرها المثبط في نمو ستة انواع بكتيرية . المستخلص المائي كان اوضح تأثيرا من مستخلص الكلوروفورم على كل انواع البكتيريا المدروسة لاسيما بكتيريا *Staphylococcus aureus* (منطقة التثبيط 21ملم عند التركيز 70 ملغم/مل) من البكتيريا الموجبة لصبغة كرام وبكتيريا *Klebsiella pneumoniae* (منطقة التثبيط 20مم عند التركيز نفسه) من البكتيريا السالبة لصبغة كرام ونمو كل الانواع البكتيرية ثبط بدرجات مختلفة تختلف بأختلاف تركيز ورق الحنة مما يعني امتلاك نبات الحنة الكفاية الضد مايكروبية .

هذه النتائج تثبت النشاط الضد بكتيري لورق الحنة وتدعم الاستعمال التجاري للنبات في علاج الاصابات البكتيرية .