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Studying the Effect of Locally Henna Plant *Lawsonia inermis* on *Pseudomonas aeruginosa*



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ABSTRACT

The study included (10) isolates of *Pseudomonas aeruginosa* were obtained from high studies laboratory in college of science / Al-mustansiryah, collected from wound infections. Isolates in current study were tested for antimicrobial susceptibility against numbers of antibiotic and these isolates were showed high resistance to Amoxicillin, Cefotaxime, ceftazidime 100% and 80% for Ciprofloxacin. Also, biofilm production from *pseudomonas aeruginosa* studied and results showed that 30 % of isolates had high biofilm production; also 30% were moderated biofilm producer, while 40% of isolates were no biofilm producer. The effect of henna plant *Lawsonia inermis* were studied, antimicrobial effect of methanolic, alcoholic and aqueous extract of henna against *Pseudomonas aeruginosa* in current study were investigated in vitro by using well diffusion method, methanolic extract of henna showed clearly effect of the concentration 50%, 75%, 100% against Isolates, while alcoholic and aqueous extract of henna did not give any effect on isolates.

INTRODUCTION

Lawsonia inermis (henna) is a tropical and subtropical saplin, growing in north Africa, middle east and Indian subcontinent, the powder crafted of dried crushed leaves is called henna.[1] Medicinal plant as henna is used as antibacterial compounds which have high influence, low toxicity, inexpensive and more efficient.[2]

fundamental uses of henna are as a refreshing agent, astringent, antifungal and antibacterial, herb for the skin and hair, it has also been used as a dye and conservator for hair, skin, fingernails as well as leather and clothes.[3,4]

Pseudomonas aeruginosa is an opportunistic gram negative pathogen which causes abstruse infections predominantly of the lower respiratory tract, wounds and urinary tract. [5]

Some virulence factors advantage this pathogens infection, such as forming of pyocyanin, hemolysin, gelatinase and biofilm, which make increasing tissue damage and protecting *Pseudomonas aeruginosa* against the recognition of the immune system and the action of antibiotics. [6]

MATERIALS AND METHODS

Bacterial isolates:

Reference microbial strains obtained from high studies laboratory in college of science. Al-mustansiryah. Ten isolates of *pseudomonas aeruginosa* were collected from wound infections, these isolates were identified by using culturing, morphological, biochemical tests and api 20E system.

Antimicrobial susceptibility test:

According to the [7] disk diffusion test had done for cefotaxime (30ug), ceftazidime (30ug), amoxicillin (25ug) and ciprofloxacin (5ug).

Collection of plant:

Leaves of *Lawsonia inermis* from private gardens in Baghdad city were collected.

Preparation of plant extract:

Three different types of extracts involve methanol extract; aqueous extract and alcohol extract to determine the *in vitro* antimicrobial activity of *Lawsonia inermis* were prepared.

Dried leaves of henna were ground to fine powder mechanically in electric grinder. Powder leaves (10g) were added in three flasks of (100ml) volume and (50ml) of each solvent was added to each flask separately. The flask was kept in incubator at 37C for overnight, the contents of flask were first filtered through the layers of muslin cloth and then through Whatman filter paper, the preparation of plant extract was done at different concentration (100%, 75%, 50%, 25%).

Antimicrobial activity of Henna:

Antimicrobial activity of *Lawsonia inermis* extract was performed by using agar well diffusion method with different concentration of leaf extract [8] the test was performed with each bacteria isolates and mean zone of inhibition was recorded.

Biofilm formation:

The phenotypic method was used for detection of biofilm production of all isolates by Congo Red Agar method (CRA) according to [9].

The CRA medium was prepared with 37g/1LBHI broth 50g/1sucrose, 10g/1 agar and 0.8g/1congo red. Congo red stain was prepared as a concentrated aqueous solution and autoclaved at 121 °C for 15 min separately from other medium constituents' and was then added when the agar had cooled to 55 °C. Plates were inoculated and incubated at 37C for 24hrs.

The plates were inspected for the color of the colonies at 24 and 48 hrs. A positive result was indicated by black colonies whereas non producing strains developed red colonies, the Congo red dye directly interacts with certain polysaccharides forming colored complex or, more likely some metabolic changes of the dye to form a secondary product could play a more important part in the formation of dark colonies. [10] For colonies color evaluation according to [11] black and Bordeaux almost black were classified as biofilm – producers while pink and red as non – biofilm producing strain.

RESULTS AND DISCUSSION

In current study results showed that all bacterial isolate were found to be highly resistant to Amoxicillin, ceftazidime and cefotaxime 100% (table 1), these results come in agreement with other studies [12] which pointed that *Pseudomonas aeruginosa* usually considered multi – drug resistance and it develops resistance by various mechanisms like efflux pumps, production of β -lactamase, aminoglycoside modifying enzymes and decrease the permeability of outer membrane. While *Pseudomonas aeruginosa* 8 and 9 isolates did show sensitive to ciprofloxacin and the rest isolates of *Pseudomonas aeruginosa* were resistant 80% to ciprofloxacin. Bacterial contamination of wounds is an important cause mortality rapidly appearing nosocomial pathogens and the problem of multi-drug resistance require recurring review of antibiogram pattern of organism isolated in wounds [13], in our results we were showed correlation between biofilm producer and antibiotics resistance for *Pseudomonas aeruginosa* isolates.

Table (1): Antibiotics sensitivity of *Pseudomonas aeruginosa* isolates

Isolates	Cefotaxime	Ceftazidime	Ciprofloxacin	Amoxicillin
<i>Pseudomonas aeruginosa</i> 1	R	R	R	R
<i>Pseudomonas aeruginosa</i> 2	R	R	R	R
<i>Pseudomonas aeruginosa</i> 3	R	R	R	R
<i>Pseudomonas aeruginosa</i> 4	R	R	R	R
<i>pseudomonas aeruginosa</i> 5	R	R	R	R
<i>Pseudomonas aeruginosa</i> 6	R	R	R	R
<i>Pseudomonas aeruginosa</i> 7	R	R	R	R
<i>Pseudomonas aeruginosa</i> 8	R	R	S	R
<i>Pseudomonas aeruginosa</i> 9	R	R	S	R
<i>Pseudomonas aeruginosa</i> 10	R	R	R	R

Pseudomonas aeruginosa exemplarily makes a biofilm which is a structured consortium of bacteria subsumed in a self-produced polymer matrix consisting of polysaccharide, protein and

DNA which cause chronic infection because it is show increased tolerance to antibiotics and make treatment complicated and also resistance to phagocytosis [14].

Congo red method showed that 30% of isolates had high biofilm production, also 30% of isolates were moderated formed of biofilm, while 40% of isolates were no biofilm producer. (Table 2, Fig 1)

Table (2): Isolates producing biofilm

<i>Pseudomonas aeruginosa</i> isolates	Biofilm producing
<i>Pseudomonas aeruginosa</i> 1	High biofilm producer
<i>Pseudomonas aeruginosa</i> 2	Moderate biofilm producer
<i>Pseudomonas aeruginosa</i> 3	No biofilm producer
<i>Pseudomonas aeruginosa</i> 4	No biofilm producer
<i>Pseudomonas aeruginosa</i> 5	Moderate biofilm producer
<i>Pseudomonas aeruginosa</i> 6	High biofilm producer
<i>Pseudomonas aeruginosa</i> 7	No biofilm producer
<i>Pseudomonas aeruginosa</i> 8	High biofilm producer
<i>Pseudomonas aeruginosa</i> 9	Moderate biofilm producer
<i>pseudomonas aeruginosa</i> 10	No biofilm producer



Figure (1) *Pseudomonas aeruginosa* 6 producing biofilm on Congo Red Agar (CRA)

The methanol extract of Henna showed its effect on *Pseudomonas aeruginosa* 1 isolate of the concentration (50%,75%, 100%) where the diameter of inhibition zone (16mm,14mm,10mm) of the concentration respectively, while the concentration 25% of the extract did not show any effect.

Methanol extract of Henna had effected on the isolate *Pseudomonas aeruginosa* 9 of the different concentration 75% and 100% where the diameters of inhibition zone 20mm, 12mm, respectively while the extract of concentration 25% and 50% did not affect on it.

The results also showed that the isolate *Pseudomonas aeruginosa* 4, only the concentration 100% of the methanol extract of henna effect on it while the concentration 75%, 50% and 25% did not show any effect on this isolate. The methanol extract of henna did not appear any effect upon the all concentration 25%, 50 %, 75% and 100% on the rest of isolates.

The aqueous and alcoholic extract of henna did not give any effect on isolates in current study.

Fig (2)



Figure (2) the effect of methanol extract of Henna on isolates

Some studies proposed that henna has a wide spectrum of antimicrobial activity including antibacterial, antiviral, antimycotic and antiparasitic activities with the over increasing resistant strain of microorganisms to the already available and synthesized antibiotics. [15]

In [16] the plant extracts showed high activity against most microorganisms tested except for the aqueous *L.inermis* which showed lowest antimicrobial effect on most bacteria tested, also [17] refers that the aquatic extract of henna had no effect on *E. coli* and *Pseudomonas SP*.

The extracts of tested plant showed a great activity in inhibiting growth of bacteria and fungi, probably due to the presence of active ingredients that inhibit bacterial and fungal growth. Henna contains lawsone in about 0.5 to 1.5 % of its ingredients, lawsone(2-hydroxynaphthoquinone) is the superior constituent responsible for the dyeing properties of the plant, also henna includes mannite, tannic acid, mucilage and Gallic acid.[18]

Also, antimicrobial activity may be due to numerous free hydroxyl ions that have the capability to combine with carbohydrates and proteins in the bacterial cell wall, they may get attached to enzyme sites rendering them inactive [16,19].

Our results are suitable with the results of [20] which showed that the antibacterial activity of henna extracts, alcoholic and oily extracts were more effective than the aquatic extract, and this may be due to the lack of the solvent properties which plays an essential function in antibacterial efficacy [21] other explorations showed that henna is effective against different microorganisms especially against *Pseudomonas aeruginosa* [22,23].

CONCLUSION

Locally *Lawsonia inermis* plant extracts showed high activity in inhibiting the growth of microorganisms and this supports traditional use of plant in therapy of bacterial infections after purify the active principle and evaluate for therapeutic enforcement.

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