

## Biological Effect of *Lawsonia Inermis* Plant

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### ABSTRACT

This study was compared the antimicrobial effectiveness of *Lawsonia inermis* plant extract (alcoholic, aqueous and silver nanoparticles) on the growth of different types of *Gve+* bacteria and *Gve-* bacteria at concentration 80 mg /L. It was noted that the (alcoholic, hot and cold water) extract have highest impact recorded in the growth of bacteria *S.aurous* with inhibition zone (26mm) followed by the bacteria *P.aeruginosa* at inhibition zone reached (22mm) and finally the yeast *C. albicans* at inhibition zone reached (17mm), while silver nano-particles of the plant extract the most influence on the bacteria, it gave the highest Inhibition area reached (30mm) on the growth of the *S. aurous*, followed by the inhibition area of *P.aeruginosa* reached (26mm) then *Strep. pyogein* (25mm), and finally the yeast *C. albicans* reached (18 mm). With regard to the complex (alcoholic extract and antibiotic Amoxylein) its effects has varied upon microorganisms, there is a synergistic effect on the growth of the yeast *C. albicans* at inhibition zone (30mm) while it has been observed inhibitory effect of this complex on the growth of *p. aeruginosa* with inhibition zone amounted to (15mm), but did not show any significant impact on the growth of both bacteria (*K. pneumonia*, *E.coli* *Strep. Pyogein* & *P. aeruginosa*), while the (alcoholic extract and antibiotic Erythromycin) showed inhibitory and synergistically effect on each of the bacteria (*E.coli*, *S. aurous*, yeast *C. albicans* and *P. aeruginosa*) respectively. According to obtained results the antibiotic Erythromycin shows higher inhibitory effect than antibiotic Amoxylein. It has also been investigated the lower values of absorbance at the wavelength of 260 nm with increasing the concentration of crude alcoholic extract, this reveled the ability of henna extract in the removal of free radicals liberated from photolysis of hydrogen peroxide compound .

### الخلاصة

في هذه الدراسة تمت المقارنة بين الفعالية المايكروبية للمستخلصات الكحولية ، المائية وجزيئات الفضة النانوية المتكونة بواسطة نبات الحنة *L. inermis* ، في نمو انواع مختلفة من البكتريا الموجبة والسالبة لصبغة كرام بتركيز 80 ملغم/لتر<sup>1</sup> ولوحظ ان المستخلص الكحولي والمائي الحار والبارد سجل اعلى تأثير في نمو بكتريا المكورات العنقودية *S.aureus* (26ملم) تليها بكتريا الزوائف الزنجارية *P. aeruginosa*. بقطر منطقة تثبيط بلغ ( 22ملم) واخيرا خميرة *C. albicans* بقطر منطقة تثبيط بلغ (17ملم) ، بينما اظهرت جزيئات الفضة النانوية للنبات تأثير اكبر على البكتريا قيد الدراسة وبصورة متباينة فقد اعطى اعلى منطقة تثبيط بلغت (30 ملم) في نمو البكتريا *S.aurous* ويليهما في ذلك البكتريا *p. aeruginosa* بمنطقة تثبيط بلغت (26ملم) ثم في بكتريا الزوائف الزنجارية *Strep. pyogein* (25ملم)، واخيرا خميرة *C. albicans* بلغ (18ملم). وفيما يخص معقد (المستخلص الكحولي والمضاد الحيوي Amoxicilin) فقد تباين تأثيره على الاحياء المجهرية فقد لوحظ بان هنالك تأثير تازري له في نمو الخميرة *C. albicans* بمنطقة تثبيط بلغت (30 ملم) في حين لوحظ تأثير تثبيطي للمعقد في نمو الزوائف الزنجارية بمنطقة تثبيط بلغت (15ملم) ولكن لم يظهر اي تأثير يذكر في نمو كل من البكتريا (*K. pneumonia*) فقد اظهرت تأثيرا تازري واضح لكل من بكتريا المعوية و المكورات العنقودية و الخميرة *C. albicans* و الزوائف الزنجارية على التوالي. وقد لوحظ ان المضاد الحيوي Erythromycin اعطى تأثير تثبيطي اعلى من المضاد الحيوي Amoxicilin . ولقد لوحظ من خلال انخفاض قيم الامتصاصية عند الطول الموجي (260 نانوميتر بزيادة تركيز المستخلص المستخدم قابلية هذا المستخلص الكحولي الخام في ازاحة الجذور الحرة المتحررة من التحلل الضوئي لمركب بيروكسيد الهيدروجين .

### INTRODUCTION

The *Lawsonia inermis* (henna) plant belongs to the family Lythraceae ,it has coloured leaves[1], it is a glabrous branched shrub ,and it is a small brown bark tree [2]. Henna has chemical compounds like, (carbohydrates, proteins, flavanoids, tannins and phenolic compounds, alkaloids, quinones, coumarins, and fatty acids). The plant has many uses: analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-

inflammatory, antibacterial, antimicrobial, antifungal, antiviral, , antitrypanosomal, , antioxidant, antifertility, and anticancer properties. Leaves of henna are useful to minimize the severity of many medical problems like dysentery, diseases of the spleen, and syphilitic eye infection [3]. Antimicrobial activity of the phenolic compounds found in henna were inhibited the bacterial growth of both *Gram +ve* and *Gram -ve* bacteria [4]. The (Gallic acid) which present in henna leaves to the

extent of 5-6 % [5]. These studies showed antimicrobial activities of *L. inermis* against different bacterial species. *L.inermis* is a red-orange substance which concentrated in the leaves[1]. Leaves of henna stain the skin with red orange color when they are smashed with an acidic liquid, which then gradually moves into the outer layer of the skin and binds to the proteins that create a fast stain. The aim of this research is to find the antimicrobial activity of henna extracts (alcoholic, hot & cold-water, nanoparticles) against different species of bacteria.

## MATERIALS AND METHODS

### Plant Samples

The powder of Henna (*L. inermis*) plant was obtained from the local market of Baghdad.

### Extraction Methods

#### Extraction by Ethanol

The powder of Henna plant (50gm) was extracted by boiling (300ml) ethanol via Soxhlet for 7 hours. The extract was filtered by Whatman filter paper using a Buchner funnel under vacuum. A rotary evaporator under vacuum at 40°C was used to filtrate the extract of henna, which then stored and kept in a dark refrigerator at 4°C in a glass container [6].

#### Extraction by Hot Water

The powder of Henna plant (50 gm) was soaked in (300ml) hot water for 5 hours. The extract was filtered by Whatman filter paper using a Buchner funnel, which then was kept in an oven at (50°C) for drying[6].

#### Extraction by cold Water

The powder of Henna plant (50 gm) was soaked in (300ml) cold water for 5 hours. The extract was filtered by Whatman filter paper using a Buchner funnel, which then was kept in an oven at (50°C) for drying[6].

### Bacterial strains isolation

The microbial strains are (*C.albicans*, *E.coli*, *P. aeruginosa*, *S.aureus*, *K.pneumonia*, and *Strep. pyogenis*) were obtained from ALAlweia child's hospital in Baghdad

### Assay for antibacterial activity:-

#### Preparation of inoculums:-

The test bacterial strains were inoculated into nutrient broth and were kept incubated at 37°C.

#### Antibacterial susceptibility testing:-

The agar well diffusion method was used to measure antibacterial activity of extracts of henna plant. The nutrient Agar No. 2 medium (Hi-Medium) was inoculated with 200 µl of the inoculums ( $1 \times 10^8$  cfu/ml), when temperature of media reached to 40-42°C the media was poured into the Petri plates. After the media

was solidified, wells were made in the plates by using a cup-borer (6 mm). The well was filled with 100 µl of the extract. For each bacterial strain wells of controls were filled with distilled water instead of the extract. The plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured to show antibacterial activity of all Henna extract.

#### Synthesis of silver nanoparticles:-

The prepared extract (10ml) was dissolved in 90 ml of 1mM ( $\text{AgNO}_3$ ) solution and incubated at room temperature. The synthesis of silver nanoparticles was indicated by change in color into brown [7].

#### Detection of Alkaloids

The addition of six drops of Dragendorff's and Mayer's reagent to 2ml plant extract in test tube and then mixing. The formation of granular white color indicates the presence of alkaloids [8].

#### Detection of Flavanoids

Five ml of dilute ammonia solution were added to each extract followed by addition of conc.  $\text{H}_2\text{SO}_4$ . The formation of yellow color indicates presence of flavanoids [9].

#### Detection of Tannins

50ml of plant extract was filtered; lead acetate 1% was added. Formation of gelatinous precipitate indicates presence of tannins [10].

**Antioxidant capacity assay:** - Free radical scavenging activity were measured by using two phases:-

#### Prepare standard DNA solution:-

DNA sample was dissolved in a solution consisting of 0.0015 Molar of sodium chloride and 0.00015 M of sodium citrate at final concentration (5) µg / ml of DNA at pH=7.

#### Investigate the effectiveness of the plant extract on the DNA molecule outside the living body: -

DNA solution prepared from above assay has a range  $A_{260} / A_{280}$  absorbance equal to 1.87, which treated with five concentrations of ethanolic extract (0.0, 0.1, 0.2, 0.3, and 0.4) µg / ml by mixing one size of henna extract (0.5 ml) with same size of standard solution of DNA, then incubated at 37 ° C for 10 minutes. At 260 nm wavelength the effect of the extract on the DNA molecule has been detected [11].

#### UV-Vis Spectra and IR analysis

The Absorption was measured by using UV-Visible spectrophotometer at a resolution of 1 nm. The UV-Visible spectra of synthesized AgNPs showed absorption peak at 400 nm that is specific for AgNPs. UV-Visible spectra of AgNPs was shown in (Figure 1) [12].

## RESULTS AND DISCUSSION

The present study was appeared that ethanolic extract have higher activity in phytochemical analysis of (Tannins , Alkaloid, Flavanoids ,Quinones) than cold and hot water extract, this result due to organic solvent more dissolved than other solvents , as publicized in next Table 1.

**Table 1 . Results of phytochemically test of *L. inermis* extracts.**

Phytochemical compounds	Extracts		
	cold water	ethanolic	Hot water
Tannins	-	+	+
Alkaloid	-	+	-
Flavanoids	+	+	+
Quinones	+	+	+

Positive +, Negative -

**Antimicrobial test of (ethanolic, hot & cold water and silver nano particles) *L. inermis* extracts.**

Antibacterial activity of aqueous (hot, cold), ethanolic and nano extracts of *L. inermis* were tested against of some type of bacteria in Table (2,) and figure (1, 2, 3&4) show the results of antibacterial activity of *L.inermis* extracts. The highest antibacterial potency was observed for ethanolic extract with zone of inhibition. Extract of ethanol was clearly superior in bioactivity as compared to that of. aqueous extract. The maximum inhibition zone was found in ethanol extract concentration and it was 26mm in the bacterial isolate of *S. aurous*, while the least inhibition zone in the ethanol extract was 17mm for the *C. albicanes*.while the highest zone of inhibition extract of aqueous extract in the bacterial isolate of *S. aurous* was 25 mm, least inhibition zone in the *K.pneumonia* was 15mm. Results of this study indicate that ethanolic extracts had more activity against bacteria this result is in agreement with [13], whom noted that, alcohols are most superior solvent for extraction of antimicrobial substances as compared to water. It was noted that nano extract of henna represent higher inhibition zone than ethanolic and aqueous extract ,where it was found highest antibacterial potency in *S. aurous* 30mm and the lower inhibition zone in *C. albicanes* 18mm.The above results showed antibacterial activity of *L. inermis* extracts on the *Gv+e* was higher than *Gv-e*. These differences among bacteria species may also be the result of the cell wall in gram-positive bacteria of a single layer, whereas the *gram-negative* cell wall is multi-layered structure and the yeast cell wall is quite complex.

Table 3.shows Antimicrobial effect of Amoxylein and Erythromycin and,it was found that Antibiotic Erythromycin with ethanolic extract be more effective than Amoxylein against isolates of bacteria. In this study, the influence of amoxicillin and erythromycin and there ligands on their respective antibacterial activities was investigated. While erythromycin inhibited more organisms than amoxicillin. The combined Antibiotic

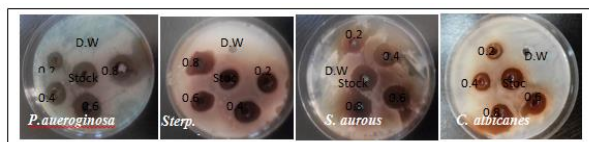
erythromycin with ethanolic extract produced varied inhibition zones when tested against each bacterial isolate. The inhibition zones obtained of *K. pneumonia* (30mm) was bigger than obtained from Antibiotic Amoxylein with ethanolic extract.

**Table 2. Antimicrobial effect of types extract leaf of *L. inermis* Concentration mg mL<sup>-1</sup>.**

Micro-organisms	inhibition zone in mm			
	hot water	cold water	ethanolic	Silver nanoparticles
<i>E. coli</i>	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	15mm	22mm	26mm
<i>Sterptococcus pyogein</i>	17mm	20mm	20mm	25mm
<i>Staphylococcus aurous</i>	25mm	25mm	26mm	30mm
<i>Candida albicanes</i>	-	-	17mm	18mm
<i>Klebsseila pneumonia</i>	16mm	15mm	-	-

**Table 3. Antimicrobial effect of saturated (Antibiotics and extract) complex on growth of bacterial strains.**

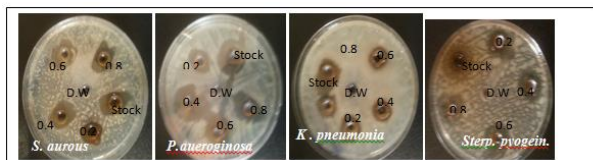
Microorg anisms	Antibiotics		Saturated Antibiotic with extract	
	Erythro mycin	Amoix cylin	Erythro mycin	Amoix cylin
<i>E. coli</i>	27mm	-	28mm	-
<i>P.aeruginosa</i>	-	-	15mm	15mm
<i>Sterp. pyogein</i>	22 mm	-	25 mm	-
<i>S. aurous</i>	22 mm	-	25 mm	-
<i>C. albicanes</i>	-	-	30mm	30mm
<i>K. pneumonia</i>	30mm	-	30mm	-



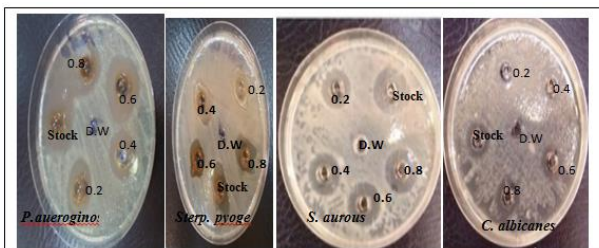
**Figure 1. The Antimicrobial activity of ethanolic extract leaf of *L. inermis* plant.**



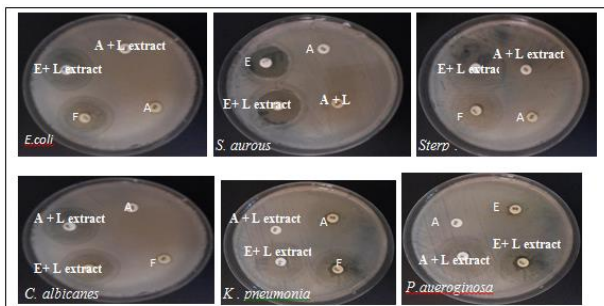
**Figure 2. The Antimicrobial activity of hot extract leaf of *L. inermis* plant.**



**Figure 3. The Antimicrobial activity of cold extracts leaf of *L. inermis* plant.**



**Figure 4. The Antimicrobial activity of nano extracts leaf of *L. inermis* plant.**

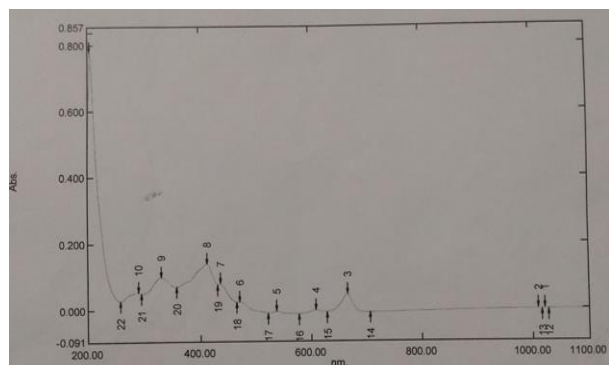


**Figure 5. Antimicrobial effects of Ligands (Erythromycin with ethanolic extract & Amoxicillin with ethanolic extract *L. inermis*) on growth of tested microbes.**

E= Erythromycin only, E+L extract = Erythromycin with ethanolic extract, A= Amoxicillin only, A+L extract = Amoxicillin with ethanolic extract.

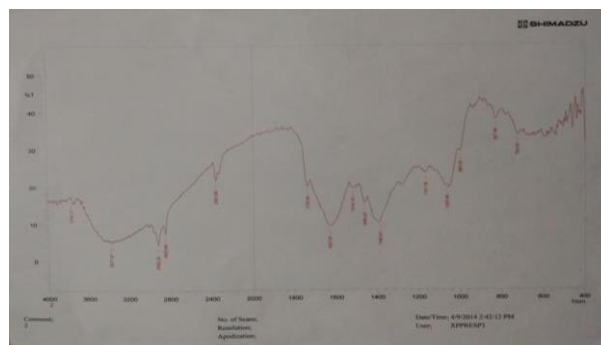
**UV-Vis spectrophotometer and Infrared (IR) spectroscopy analysis**

The Ag nanoparticles were characterize by means of UV-Vis spectrophotometer, which is lone of the most widely use methods for characterization of Ag nanoparticles [14]. Which is shown in Figure 6.



**Figure 6. UV-Vis absorption spectra of Ag nanoparticles suspension synthesize via *L. inermis*.**

Spectroscopy is the study of infrared light interacting with a molecule. It is used by chemists to conclude practical groups in molecules. The next figure represents the IR spectrum of *L. inermis* green silver nanoparticles showed absorption peak at 29 02.25 cm-1 represented to C-H aromatic group, at 2840.88 cm-1 corresponding to C-H alphatic group. Addition peaks were at 1739.85 represented to C=O alphatic ester group, at 1701.27 corresponding to C=O Lactame.



**Figure 7. Spectrum peak pick report of ethanolic leaf *L. inermis* extract.**

**Effect of plant extract in the removal of free radicals from the DNA solution.**

The effect of different concentrations of ethanolic extract of *L. inermis* upon pure molecule DNA that prepared in a final concentration (5) Mcgm / ml, have been shown in (Table 4-A groups).The result was observed increase in absorbance with increase in the concentration of ethanolic extract, and this showed that the plant extract of henna have enzyme DNase activity. The results for B Group's represents the ability of plant to remove free radicals from the hydrogen peroxide compound with concentration (5-10) Molar ,which indicate by decreasing the values of absorbance at the wavelength of (260 nm) upon increasing the concentration of henna extract.

**Table 4. Effect of different concentrations of the henna leaves extract on the pure DNA molecule and the effect of this extract in the removal of free radicals.**

Plant extract concentration $\mu\text{g/ml}$	(A) H2O2 +DNA	(B) Ethanolic extract+DNA+H2O2
0.0	0.206	0.3
0.1	0.23	0.29
0.2	0.25	0.26
0.3	0.27	0.25
0.4	0.29	0.20

**CONCLUSION**

It is obvious that henna leaves extract has a benefit as an antimicrobial. Agent against the different type of bacteria. According to the results that have been obtained from this study, all henna extracts have antioxidant activity by the ability of plant to remove free radicals from the hydrogen peroxide compound.

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