**Biological effect of Lawsoniainermis plant**


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**ABSTRACT**

This study was compared the antimicrobial effectiveness of *Lawsoniainermis* 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.aeroginosa* 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.aeroginosa* 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. aeroginosa* with inhibition zone amounted to (15mm), but did not show any significant impact on the growth of both bacteria (*K. pneumonia, E.coli*), 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. aeroginosa*). 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 revealed the ability of henna extract in the removal of free radicals liberated from photolysis of hydrogen peroxide compound.

**INTRODUCTION**

The *Lawsoniainermis* (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

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**الخلاصة**

في هذه الدراسة تم استخراج منازل في فروع النباتات من النبات المركب *L. inermis*، الذي يتميز بالفضلة النباتية، 80 ملليمتر. تم تجربة أفضل من النباتات المركبة *L. inermis*، والتي يتميز بالفضلة النباتية، 80 ملليمتر. تم تجربة أفضل من النباتات المركبة *L. inermis*، والتي يتميز بالفضلة النباتية، 80 ملليمتر. تم تجربة أفضل من النباتات المركبة *L. inermis*، والتي يتميز بالفضلة النباتية، 80 ملليمتر.

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**Mots clés:**Effet biologique, *Lawsoniainermis*.
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 +veand Gram -vebacteria [4]. The (Gallic acid) which present in henna leaves to the extent of 5-6 % [5]. These studies was 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 a acidic liquid, which then gradually moves into outer layer of the skin and binds to the proteins that creating a fast stain. The aim of this research to find 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 local market of Baghdad.

Extraction Methods

Extraction by Ethanol
The powder of Henna plant (50gm) was extracted by boiling (300ml) ethanol via soxholet 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 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 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. pyogen ) 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 (1x10³cfu/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 wells was filled with 100 μl of the extract. For each bacterial strain well 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 (AgNO3) solution and incubated at room temperature. The synthesis of silver nanoparticles was indicated by change the color into brown [7].

Detection of Alkaloids
The addition of six drops of Dragendroofs 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.H2SO4. The form 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 consist 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 have range A260 / A280 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 wave length the effect of the extract on the DNA molecule have 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 was showed absorption peak at 400 nm that is specific for AgNPs. UV-Visible spectra of AgNPs was shown in (Fig. 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.

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>extracts</th>
<th>cold water</th>
<th>ethanolic</th>
<th>Hot water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

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. albicans. 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 15 mm. 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. albicans 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-1.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Inhibition zone in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hot water</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>-</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>-</td>
</tr>
<tr>
<td>C. albicans</td>
<td>-</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Antibiotics</th>
<th>Saturated Antibiotic with extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>28 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>15 mm</td>
<td>15 mm</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>20 mm</td>
<td>25 mm</td>
</tr>
<tr>
<td>S. aureus</td>
<td>25 mm</td>
<td>25 mm</td>
</tr>
<tr>
<td>C. albicans</td>
<td>30 mm</td>
<td>30 mm</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>30 mm</td>
<td>30 mm</td>
</tr>
</tbody>
</table>
Fig. 1. The Antimicrobial activity of ethanolic extract leaf of *L. inermis* plant.

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

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

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

Fig. 5. Antimicrobial effects of Ligands (Erythromycin with ethanolic extract & Amoixycin with ethanolic extract *L. inermis* on growth of tested microbes.

E= Erythromycin only, E+L extract = Erythromycin with ethanolic extract, A= Amoixycin only, A+L extract = Amoixycin 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 fig. 6.

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 at1739.85 represented to C=0 alphatic ester group, at 1701.27 corresponding to C=0 Lactame.

Fig. 6. UV-Vis absorption spectra of Ag nanoparticlelessuspension synthesize via *L. inermis*.

Fig. 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 hennaleaves extracton the pure DNA molecule and the effect of this extract in the removal of free radicals.

<table>
<thead>
<tr>
<th>Plant extract concentration μg/ml</th>
<th>(A) H2O2 +DNA</th>
<th>(B) Ethanol extract+DNA+H2O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.206</td>
<td>0.3</td>
</tr>
<tr>
<td>0.1</td>
<td>0.23</td>
<td>0.29</td>
</tr>
<tr>
<td>0.2</td>
<td>0.25</td>
<td>0.26</td>
</tr>
<tr>
<td>0.3</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>0.4</td>
<td>0.29</td>
<td>0.20</td>
</tr>
</tbody>
</table>

CONCLUSION
It is obvious that hennaleaves 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.

REFERENCES


