

Evaluation of anti-bacterial activity of the aquatic henna leaves extract in Hilla City, an *in vitro* study, Iraq

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ABSTRACT

Henna leaves have been amassed and selected for antimicrobial towards some human pathogens. The present investigation has been undertaken to find out the effectiveness of aqueous extracts of *Lawsonia inermis* against some human pathogenic microorganism as well as compare it with wide spectrum antibiotic ciprofloxacin.

In the present study; henna leaves had been extracted with aqueous extraction method. The isolated bacteria were diagnosed based totally on cultural, morphological and biochemical characteristics, hence, the remotes bacterial isolates were as *S.aureus*, *S. epidermidis*, *S. saprophyticus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus fecalis*, *Streptococcus agalactia*, *P.aeruginosa*, *P. fluorescences*, *Salmonella typhi*, *Salmonella typhimurum*, *Proteus merabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Enterobacter spp*, *Acinetobacter*, *Escherichia coli*, *Serratia spp*. The antibacterial activity of the Henna leaves aqueous extract was compare it with wide spectrum antibiotic ciprofloxacin.

The result shows high antibacterial activity of the *Lawsonia inermis* aqueous extract against tested bacteria with higher a significant different than obtain by ciprofloxacin antibiotics.

Conclusions: *Lawsonia inermis* exhibit marked antimicrobial activity against bacteria. Based on the results it can be concluded that they can inhibit bacterial growth more than ciprofloxacin antibiotics.

KEY WORDS: anti-bacterial activity, aquatic henna leaves extract.

1. INTRODUCTION

The handiest species in the genus *Lawsonia* in the own family Lythraceae "*Lawsonia inermis*" is involved herbal medicine that used for a while. This flowering plant, 2-6m in top. Produces a burgundy dye molecule, lawsone which has an affinity for bonding with protein so, it extensively talking used to dye pores and skin, hair, fingernails, leather-based, silk and wool. Lawsone, is in the main concentrated in the leaves. Leaves of henna plant are whole, opposite, sub-sessile, oval-usual and smooth. Leaves have period of two–3 cm with 1–2 cm width. Henna shrub is especially branched and has greyish-brown barks. The principle makes use of henna are as a cooling agent, astringent, anti-fungal and anti-bacterial herb for the pores and skin and hair. It has also been used as a dye and preservative for hair, pores and pores and skin and fingernails in addition to leather-based completely and clothes. Its center chemical additives are 2-hydroxynaphthoquinone (lawsone), mannite, tannic acid, mucilage and gallic acid. Out of these components, the principle one is 2-hydroxynaphthoquinone (lawsone). About zero.five-1.5% of henna is fabricated from lawsone. Its bioactive feature is idea to be because of its excessive protein binding capability. In traditional medication, henna plant is used to address many illnesses like oedema, bronchitis, menstrual disease, rheumatism, hemorrhoids or perhaps in jaundice, leprosy, ache, spleen boom, dysentery and pores and pores and skin issues (eight). Henna moreover can be used as an astringent and antihemorrhagic agent and is also stated for its hypotensive, cardio inhibitory and sedative outcomes. Further, henna is said to expose a few awesome residences which incorporates hypoglycemic (nine), immunostimulant, hepatoprotective (eleven), anti-inflammatory, tuberculostatic, anti-maximum cancers and antioxidant houses.

Aim: The present investigation has been undertaken to find out the effectiveness of aqueous extracts of *Lawsonia inermis* against some human pathogenic microorganism such as *S.aureus*, *S. epidermidis*, *S. saprophyticus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus fecalis*, *Streptococcus agalactia*, *P.aeruginosa*, *P. fluorescences*, *Salmonella typhi*, *Salmonella typhimurum*, *Proteus merabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Enterobacter spp*, *Acinetobacter*, *Escherichia coli*, *Serratia spp* as well as compare it with wide spectrum antibiotic ciprofloxacin.

2. MATERIALS AND METHODS

Plant collection:

Preparation of aquatic extracts: The leaves of *Lawsonia inermis* were obtained from the nearby market of Hilla town – Iraq (March, 2016). The samples turned into cautiously washed beneath strolling faucet water observed by using sterile distilled water after which air dried for two days, pounded. The usage of a mixer grinder and stored in hermetic bottles. Education of aquatic extracts (Aqueous extract) become soaked 50 gram of *Lawsonia inermis* leaves via 100 ml distilled water, and allowed to face for 72 hr, and sterilized by filtration (the usage of Millipore 0.forty

five filter out paper). This extract turned into taken into consideration because the 50% attention of the extract. Oil of cardamom have been accumulated from a retail food keep. The extracts stored in sterile bottles and saved in freezer at 4°C till further use for screening of antimicrobial activity.

Bacterial Isolates: Different fifteen medical microbial isolates (eight Gram positive, 11 Gram negative) had been isolated and recognized by means of the use of traditional biochemical test and Api system (Biomeraux, France) and cultivated in natural culture, at microbiological laboratory/university of Biotechnology/ Al-Qasim green university.

In vitro Antibacterial activity testing the use of Agar nicely diffusion assay: Loop full growths from bacterial isolates had been inoculated into nutrient broth incubated at 37°C for 18 hours. The bacterial suspensions have been diluted with everyday saline. regulate the turbidity and examine with widespread tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU/ml. Cotton swab became dipped and streak into adjustment suspension the entire Mueller-Hinton agar (for all examined bacteria) floor of plates and the plates have been left for one 5-15 minutes at room temperature to dry. Media have been reduce into four wells (5mm diameter) by using cork borer and add 20 μ of the cardamom extracts solutions and oil of cardamom (The plates had been performed in triplicates). All plate of the examined organisms was then allowed to incubate at 37°C for in a single day. After 24 h of incubation, every extract turned into cited for region of inhibition for all isolates. The diameters of the region of inhibitions had been measured by means of measuring scale in millimeter (mm).

Antibacterial activity assay: The antibacterial activity became determined through agar disc diffusion. Agar plates have been inoculated with zero.1 ml broth subculture of tested organisms and become spreader with sterile an L-formed rod glass spreader. The antibiotics disks of ciprofloxacin had been upload inside the center of agar plate. (The plates had been finished in triplicates). All plate of the tested organisms changed into then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract became mentioned for quarter of inhibition for all isolates. The diameters of the region of inhibitions were measured by means of measuring scale in millimeter (mm).

Statistical evaluation: Bonferroni test endorsed with the aid of turned into used for statistical evaluation ($P \leq 0.05$) to show if there's any widespread differences among consequences of *Lawsonia inermis* extract take a look at microorganisms.

Twenty gram positive and gram negative bacteria have been used in this look at to determine the antimicrobial pastime of *Lawsonia inermis* extract. All bacterial traces have been maintained on freshly prepared blood agar. Then it's have been identified to the species degree based totally at the stander biochemical and microbiological strategies.

Antimicrobial assay: The antimicrobial activity changed into examined the usage of agar well diffusion approach. Agar plates have been swabbed with 100ml of respected broth way of life (1.5×10^8 CFU/ml), standardized by 0.5 Mac Farland) and have been kept at room temperature for 15min for absorption to take region. Wells of 6mm size have been made with sterile borer in inoculated agar plates and loaded with 100ml of plant extracts. Previous to incubation at 37°C for 24hrs. The petri dishes had been saved at room temperature for 15 min. with a purpose to promote diffusion of the extracts into the agar .all the checks were made in triplicate and the suggest diameter of inhibition zones in millimeter.

3. RESULTS AND DISCUSSION

The results were showed in the figure which revealed a higher antibacterial activity of *Lawsonia inermis* aqueous extract against most common bacterial strains in both type (Gram positive and negative bacteria) with no significant difference ($P > 0.05$) according to activity of the extract between tested bacteria. It is well established that the β - asarones found in leaf, roots and rhizomes tissues are responsible for almost all of the antimicrobial activities of the *Lawsonia inermis*. Diacylheptenoid and its Fyrosdiacrosyenin were identified as the constituent responsible for this activity. In this work, aqueous extracts of *Lawsonia inermis* inhibits the skin pathogens *S.aureus*, mutans and *P.aeruginosa* potentially. The antibacterial activity may be attributed to not only a single active principle but to a cocktail of a variety of active principles or alkaloid.

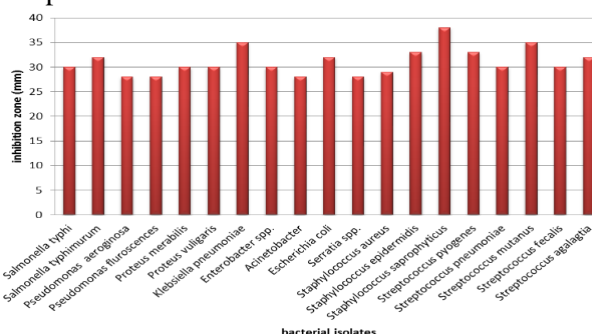


Figure.1. Antibacterial activity of *Lawsonia inermis* aqueous extract against tested bacteria

In this search, the result was compare between the result obtained by tested extract and results obtained by ciprofloxacin antibiotic (as most traditional treatment for infection caused by tested bacteria) concerning their antimicrobial activity against tested bacteria; as reveals in (figure.2).

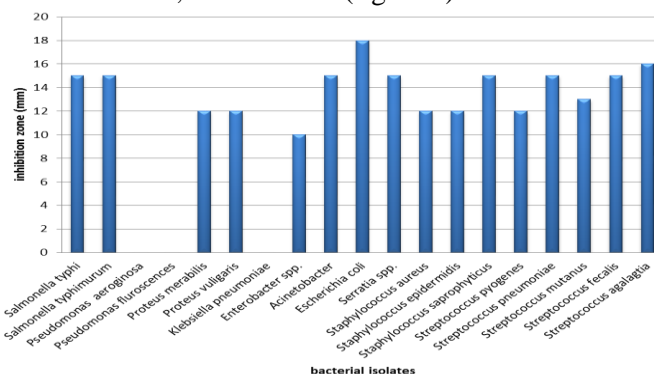


Figure.2. effect of ciprofloxacin against different types of bacteria

aqueous extracts showed more activity as antimicrobial agent against tested bacterial isolates compare to ciprofloxacin according to inhibition zone diameter and even antibiotic resistant strains (*Pseudomonas aeruginosa*, *Pseudomonas fluorescences*, *Klebsiella pneumoniae*) and less sensitivity bacteria to ciprofloxacin (*Enterobacter spp.*, *Proteus merabilis*, *Proteus vuligaris*, *Staphylococcus aureus*, *Staphylococcus epidermidis*). These bacterial strain is a mainly multi-drug resistant bacteria that are most commonly found, especially through nosocomial infections.

Considering the findings of this study and comparison with other studies in this field tea extract can be controlled growth of ciprofloxacin resistance bacterial strains which involved in this study within *in vitro* condition.

4. CONCLUSIONS

Lawsonia inermis exhibit marked antimicrobial activity against bacteria. Based on the results it can be concluded that they can inhibit bacterial growth more than ciprofloxacin antibiotics.

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