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Antimicrobial Activity of Crude Henna Extract Against Gram-positive Bacteria

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Abstract

Objectives: This study aimed to examine the effect of ethanolic extract of local Basra henna leaves on Gram-positive bacteria species. Also, to assess the antibacterial properties of henna crude extract *in vitro* and compare them with antibiotics.

Methods: In this study, *Lawsonia inermis* (henna) leaves were extracted with ethanol using the solvent extraction technique. The pathogens were isolated from wound samples obtained from hospitalized patients in two different hospitals in Duhok city. The culture of thirty isolates had been recognized by routine methods. Different concentrations of ethanol crude extract were acquired and bio-assayed *in vitro* to inhibit the growth of five human pathogenic Gram-positive bacteria. Agar well diffusion assay was used for achieving henna antibiotic activity. Moreover, an antibiotics susceptibility test was done by the disk diffusion method using the Muller-Hinton agar medium.

Results: The growth of all tested bacteria was suppressed to various degrees by increasing the concentration of the extract. The data has revealed that *Staphylococcus aureus* was more sensitive than other examined isolates, where the diameter zone of inhibition was ranging from 16–27, 14–25, and 8–18 mm for *Staphylococcus epidermidis*, *Lactobacillus* spp. and *Streptococcus pneumonia* respectively. The antimicrobial activity of henna extract indicates that it is suitable for being used as significant certain medications. Consequently, henna is active to serve as an anti-bacterial agent against multi-drug resistant Gram-positive bacteria.

Conclusion: The antimicrobial activity of henna extract indicates that it is suitable for being used as significant certain medications. Consequently, henna is active to serve as an anti-bacterial agent against multi-drug resistant Gram-positive bacteria.

Keywords: Lawsonia inermis, extract, antimicrobial activity, medicinal plant, henna

Introduction

Antibiotics' easy access and effectiveness led to overuse, particularly in livestock raising, prompting bacteria to develop resistant.[1] Excessive usage of antibiotics is damaging to human health, the environment, and the ecosystem. It might also raise the occurrences of drug-resistant pathogens.[2,3]

Nowadays, antibiotic resistance is a global main problem that is quickly increasing in both the community involved in morbidity, mortality, healthcare sectors, and hospitals.[4] This condition forced scientists to search for new antimicrobial substances. Thus, for the cure of infectious diseases, there is a necessity to develop alternative antimicrobial medicines from medicinal plants.[5] Many of today's recent and effective medications originate from traditional folk medicine to substitute artificial antibiotics.[6-8]

Medicinal plants have played an important role in ancient traditional methods of medication in numerous countries. They serve as significant raw substances for drug manufacturing because they are rich sources of bioactive compounds.[9,10] Furthermore, side effects caused by medicinal plants are less than those caused by synthetic drugs.[11] Nowadays, researchers have inspected plants with an extensive variety of secondary compounds that might be a possible source for many antimicrobial agents.[12,13]

The *Lawsonia inermis* or (Henna) is a flowering plant, 2–6 m in height. It is the sole species in the genus *Lawsonia* in the family *Lythraceae*.[14] And it is extensively grown in a variety of tropical areas in North Africa, Indian, and the Middle East subcontinent.[15] The word «henna» refers to *L. inermis*, in Arabic[16,17] and it has medicinal properties.[18,19] This plant is rich in an extensive variety of secondary metabolites, like alkaloids, tannins, terpenoids, resins, pesticides, flavonoids, and other pharmacological compounds which have confirmed it's an *in-vitro* antimicrobial agent.[20] Lawsone is a maroon dye molecule that is produced from henna.[21] This molecule has an affinity for bonding with protein and consequently has been used to dye hair, skin, fingernails, silk, wool, and leather.[22]

Recent pharmacological research on henna and its components has confirmed its anti-inflammatory, analgesic, and antipyretic effects and discovered its anti-carcinogenic potential.[23] A study by Rathi et al. 2017, found out that the extract of *L. inermis* leaves proved to own antimicrobial activity.[1] Another study done by Mohammed et al. 2006, concluded that henna leaves are used as a treatment in skin diseases in the form of a paste, where henna is used for the cure of bruises, skin inflammation, and boil burn.[24] The fungicidal and antibacterial effect of henna has long been acknowledged in earlier researches.[25-28]

In this current study, we examined the effect of ethanolic extract of local Basra henna leaves, on five Gram-positive bacteria species. Also, to assess the antibacterial properties of henna crude extract *in vitro* and compare them with antibiotics.

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Material and Methods

This study was conducted in the Microbiology Department of Nursing College, University of Duhok, Iraq, During April to November 2019.

Sample Collection and Identification

Thirty isolates (30) of Gram-positive bacteria were subjected to henna alcoholic extract. Bacterial isolates have been obtained from wound infections of patients who attend the Dermatology outpatient clinic in Azadi teaching Hospital and Burn Hospital in Duhok city, all samples were identified via routine conventional techniques.

Isolation of Organisms

Nutrient and Eosin Methylene Blue (EMB) medium used in the identification and isolation of pathogenic bacteria. Muller-Hinton agar was prepared following the manufacturer's guidelines[6] and used as an antibiogram medium in the present study. Then the obtained wound samples were inoculated into the agar plates, which were incubated for 24 hrs at 37°C.

The Plant Material

L. inermis leaves were collected from private gardens in the city of Basra in the southern part of Iraq, and identified by Professor Dr. Salim Sh. Ismael, Department of Botany, College of Agriculture, University of Duhok (Figure 1). The plant leaves have been washed thoroughly 2–3 times below the running tap and then sterile distilled water. These leaves were dried at room temperature for two days in open-air protected from direct exposure to sunlight and were afterward ground to a powder using a household electric blender. The dried powder was stored in an air-tight bottle at 28°C for additional extraction.

Extract Preparation

Fifty grams of henna powder were put in a 250 ml flask, followed by adding 100 ml of solvent (95% ethanol). The flask was then left at room temperature for 18 hrs preceding filtration. This combination was cooled and filtered through double-layered muslin fabric and then filtered by Buchner funnel and Wattman No.1 filter paper. The filtrate was concentrated under decreased pressure with an evaporator at 40°C. This crude extract was saved at 4°C until use, this extract of henna was considered as the 100% concentration. Then the concentrations (75%, 50%, and 25%) were made by diluting the concentrated extract of henna with appropriate volumes of sterile distilled water.

Antimicrobial Activity

The antimicrobial effect of the henna extract was evaluated using the disk inhibition zone method. In this method, (Kirby and Bauer, 1966),[28] the Muller-Hinton agar medium was inoculated with freshly prepared cells of bacteria to yield growth. After solidification of the agar, several sterile disks were dipped into the extract solution and placed on plates. After incubation for 24 hrs at 37°C, the antimicrobial activity was measured in the diameter of the inhibition zone formed around the disk.

At the same time, a comparison antibiotic control test was made using commercial disks (Tobramycin, Amikacin, Tetracycline, and Cefotaxime), and the diameter of the inhibition zones was measured in mm.

Results

Thirty isolates (30) of Gram-positive bacteria were collected and transferred to the Microbiology laboratory, College of Nursing, University of Duhok, and cultured on Muller-Hinton agar medium. The sensitivity testing was carried out using the disc diffusion method by Kirby-Bauer (1966).[28]

In the current study, we used henna crude extract in different concentrations (100%, 75%, 50%, and 25%) by the good diffusion method. Plant material extracted with ethanol exhibited substantial antimicrobial activity on all of the examined microorganisms, particularly at higher concentrations.

The mean diameters of the inhibition zones on Gram-positive bacteria isolates were measured in (mm) and the results were recorded, Table 1. The zone of inhibition indicated by the effect of *L. inermis* leaves against tested pathogenic bacteria is demonstrated in Figure 2.

Fig. 1 ***L. inermis*** **(Henna) leaves which were collected from private gardens in the city of Basra in the southern part of Iraq.**

Table 1. **Antimicrobial activity of *L. inermis* alcoholic extract in different concentrations on the growth of 30 isolated Gram-positive bacteria isolates**

Concentration of *L,inermis* ethanolic extract %	Gram-positive bacteria strains	No. of isolates	Average diameter of inhibition zone (mm)
100	*Staphylococcus aureus*	9 (30%)	30
75			26
50			21
25			18
100	*Staphylococcus epidermidis*	9 (30%)	27
75			23
50			19
25			16
100	*Lactobacillus spp.*	6 (20%)	25
75			21
50			17
25			14
100	*Streptococcus pneumoniae*	3 (10%)	18
75			15
50			11
25			8
100	*Streptococcus agalactiae*	3 (10%)	15
75			12
50			9
25			6
		30 (100%)	

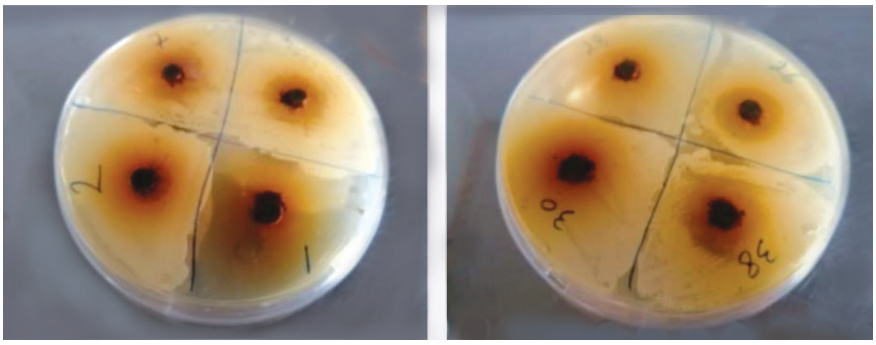

Fig. 2 **Zone of inhibition (in mm) of ethanolic extract of *L. inermis* leaves against tested pathogenic bacteria.**

Table 2. **Antibiotic sensitivity pattern with inhibition zone for each antibiotic disk used against Gram-positive bacteria isolates**

Antibiotics	Disc potency (µg)	Gram-positive bacteria strains	No. of isolates	Average diameter of inhibition zone (mm)
Tetracycline	30	*Staphylococcus aureus*	12 (40%)	28
Cefotaxime	30			24
Tobramycin	10			20
Amicacin	10			17
Tetracycline	30	*Staphylococcus epidermidis*	6 (20%)	26
Cefotaxime	30			21
Tobramycine	10			18
Amicacin	10			14
Tetracycline	30	*Lactobacillus spp.*	6 (20%)	23
Cefotaxime	30			17
Tobramycin	10			13
Amicacin	10			9
Tetracycline	30	*Streptococcus pneumoniae*	3 (10%)	16
Cefotaxime	30			13
Tobramycin	10			10
Amicacin	10			6
Tetracycline	30	*Streptococcus agalactiae*	3 (10%)	12
Cefotaxime	30			10
Tobranycin	10			7
Amicacin	10			5
			30 (100%)	

Commercial Antibiotics Sensitivity Testing

The Gram-positive bacteria isolates were also tested for their susceptibility against regularly used (commercial) antibiotics by the modified Kirby-Bauer method, Table 2. Tables 1 and 2 show the results of the comparison of inhibition diameters made by *L. inermis* extract and commonly used antibiotics for the specific microorganisms.

Discussion

The antibacterial effect of several plant extracts has been proved earlier. The leaves of *L. inermis* are nontoxic and are used for the treatment of boils, burns, bruises, and other skin infections.[29] According to the study of Papageorgiou et al., phytochemical components of henna show antimicrobial activity only against Gram-positive bacteria while it was ineffective for Gram-negative bacteria.[30]

However, numerous reports cite the inhibitory activity of henna against Gram-negative and Gram-positive organisms. In Palestine, Elmanama et al. noted that the extracts of tested plants revealed a great activity in suppressing the growth of fungi and bacteria, possibly because of the presence of active constituents that prevent fungal and bacterial growth.[31]

Our results have shown that henna leaves extracted by ethanol increased the inhibition zone of all the tested bacteria, these findings confirmed their antibacterial activity. This is consistent with previous studies which concluded that extracts of *L. inermis* (henna) and *H. sabdariffa* (roselle) were shown to have promising antibacterial properties.[32,33]

It is represented that henna has a broad spectrum of antimicrobial activity including antimycotic, antiparasitic, antiviral, and antibacterial activities. With the ever-increasing strains of microbes to the already synthesized and accessible antibiotics, the naturally available henna might be a potential alternative.[34] Antimicrobial activity may be due to many free hydroxyls that can combine with the proteins and carbohydrates in the bacterial cell wall as suggested by Harborne and Baxter and they attributed that to their attachment to enzyme sites rendering them inactive.[16,21]

According to the dose-response, the zone of inhibition was increased with increasing the concentration of the investigated extracts. The lowest concentrations (50 and 25 mg/ml) inhibited the microbes weakly. Conversely, for the high concentrations of plant extracts (100 and 75 mg/ml), the *L. inermis* extracts have recorded obvious inhibition activity against all tested bacteria isolates as shown in Table 1.

The concentration (100 mg/ml) of henna crude extract had the highest inhibitory effect about (30 mm) inhibition zone for *Staphylococcus aureus*, this may due to the high potential components in henna These results are in close agreement with preceding reports elsewhere using the same plant. In India, Rathi et al. indicated in their study that the ethanolic extracts of henna shown high antibacterial activity when tested against *Staphylococcus aureus*.[35]

In a comparable study by Jothiparakasam et al., they stated that between Gram-positive bacteria tested, the maximum zone of inhibition was found in ethanolic extracts against *Staphylococcus aureus* (26 mm), these results display the susceptibility of this microbe. Furthermore, statistics from similar work have noticed that *Staphylococcus aureus* is more susceptible than the other multi-drug resistant bacteria, to the employed plant extracts and antibiotics.[36]

These findings are in agreement with our data where the antibacterial activity of the phytoconstituents of *L. inermis* was active against Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* and having moderate potency on *Lactobacillus* spp. while were less potent against other tested bacteria isolates used in the present study, Table 1. Ali et al. indicated that the reason for this observation might be due to the variety of the antimicrobial compounds that have been isolated from the ethanolic extract.[37] The size of the zone diameter given from the plant extract was between 18 mm to 30 mm diameter which qualifies it to be used as an antimicrobial agent against multidrug-resistant microbes like *Staphylococcus aureus*. This proves that henna extract is active enough against *Staphylococcus aureus in vitro* and these results are compatible with that achieved from other studies.[38]

As demonstrated in Table 1, in *Streptococcus pneumoniae* and *S. agalactiae,* there was a decreased activity of the employed plant extract at the lowest concentration but as the concentration increased there was more antibacterial activity. Muhammad and Muhammad suggested that alcoholic henna extract has almost the same effect on *Streptococcus* species which they have tested in their investigation.[30] By the way, Aljamali indicated that this variation in antimicrobial effects could be because of the phytochemical dissimilarities and bacterial strains differences.[40]

Antibacterial sensitivity testing was carried out using Tetracycline, Cefotaxime, Tobramycin, and Amikacin, Table 2. When compared with antibiotics, alcoholic henna extracts showed more antimicrobial activity than antibiotics. We concluded that henna has *in-vitro* antibacterial activity against the tested pathogenic microbes. These findings have also been cited in the literature.[41,42]

Conclusion

The results of this study confirm the antibacterial activity of crude henna extract and exhibited noticeable effects when compared with commercial antibiotics. The present work showed that medicinal plants could be a potential source of new antibacterial agents. Therefore, our data proves the significance of plant extracts in the control of resistant microorganisms, which is becoming a hazard to human health.

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