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Antimicrobial activity of crude henna extract against Gram-positive bacteria

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ABSTRACT: Henna plant has been used in herbal medicine for ages, but the medical uses of this plant as antimicrobial agent had not been well discussed. In this study we aim 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. *Lawsonia inermis* (henna) leaves were extracted with ethanol via using the solvent extraction technique. The pathogens were isolated from wound samples obtained from hospitalized patients in two different hospitals in Duhok city. 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, antibiotics susceptibility test was done by the disk diffusion method using Muller-Hinton agar medium. 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 were ranging from 16-27, 14-25, and 8-18 mm for *Staphylococcus epidermidis*, *Lactobacillus* spp. and *Streptococcus pneumoniae* 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.

Keywords: *Lawsonia inermis*; Extract; Antimicrobial activity; Medicinal plant; Henna.

1. INTRODUCTION

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

Nowadays, antibiotic resistance is a global main problem which is quickly increasing in both the community involved in morbidity, mortality, health-care sectors and hospitals [2, 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 [5, 9, 10]. Furthermore, side effects caused by medicinal plants are less than that caused by synthetic drugs [1]. Nowadays, researchers have inspected plants with extensive variety of secondary compounds that might be a possible source for many antimicrobial agents [2, 12, 13].

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, 15]. And it is extensively grown in a variety of tropical areas in North Africa, Indian and the Middle East subcontinent [14]. The word "henna" refers to *L. inermis*, in Arabic [16, 17] and it has medicinal properties [16, 18, 19]. This plant is rich in an extensive variety of secondary metabolites, like alkaloid, tannins, terpenoids, resins, pesticides, flavonoids and other pharmacological compounds which has confirmed it's an in-vitro antimicrobial agent [16, 20]. Lawsone is a maroon dye molecule that is produced from henna [14, 21]. This molecule has an affinity for bonding with protein, and consequently has been used to dye hair, skin, fingernails, silk, wool and leather [14].

Recent pharmacological research on henna and its components has confirmed its anti-inflammatory, analgesic, and antipyretic effects [6, 23] and discovered its anti-carcinogenic potential [6, 22]. A study by Rathi et al., found out that the extract of *L. inermis* leaves proved to own antimicrobial activity [1]. Another study done by Mohammed et al., concluded that henna leaves are used as a treatment in skin diseases in the form of paste, where henna is used for the cure of bruises, skin inflammation and boil burn [11]. The fungicidal and antibacterial effect of henna has long been acknowledged in earlier researches [16, 24-27, 29]. 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.

2. MATERIAL AND METHODS

This study was conducted in department of Microbiology, college of Nursing, university of Duhok, Iraq, during April to November 2019.

2.1. Sample collection and identification

Thirty isolates 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.

2.2. Isolation of organisms

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

2.3. 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. The plant leaves have been washed thoroughly 2-3 times below 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.

Figure 1. *Lawsonia inermis* (henna) leaves which were collected from private gardens in the city of Basra in the southern part of Iraq.

2.4. 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.

2.5. Antimicrobial activity

The antimicrobial effect of the henna extract was evaluated using the disk inhibition zone method. In this method (Kirby and Bauer) [28], the Muller-Hinton agar medium was inoculated with freshly prepared cells of bacteria to yield a growth. After solidification of the agar, a number of 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 diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using commercial disks (Tobramycin, Amicacin, Tetracycline and Cefotaxime), and the diameter of the inhibition zones were measured in mm.

3. RESULTS

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

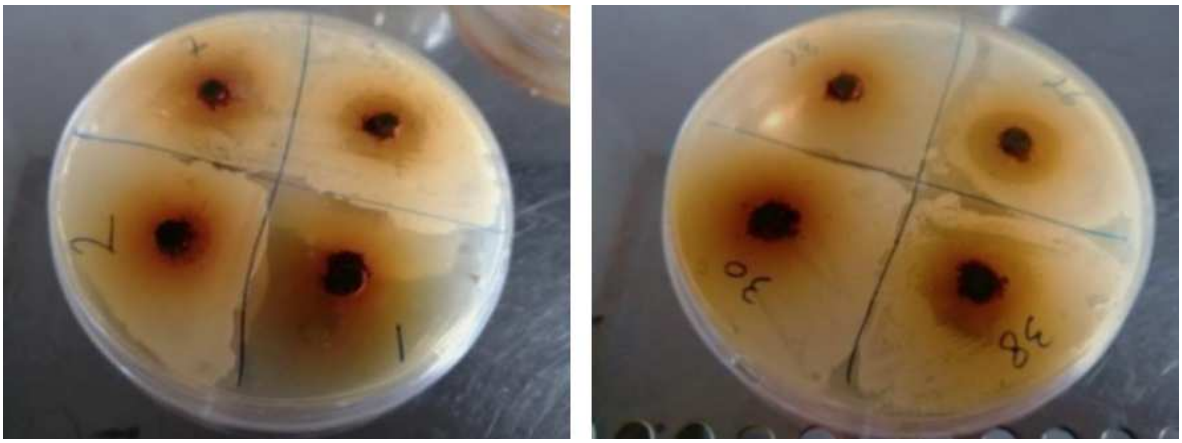

Figure 2. Zone of inhibition (in mm) of ethanolic extract of *Lawsonia inermis* leaves against tested pathogenic bacteria.

3.1. 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. Table 1 and 2 show the results of comparison of inhibition diameters made by *L. inermis* extract and common used antibiotics for the specific microorganisms.

Table 1. Antimicrobial activity of *Lawsonia inermis* ethanolic extract in different concentrations on the growth of 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

4. DISCUSSION

The antibacterial effect of several plant extracts has been proved earlier [29]. The leaves of *L. inermis* are nontoxic and are used for the treatment of boils, burns, bruises and other skin infections [30]. 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 [16].

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

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 plant 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].

The screening of plant extracts, has led to the detection of many clinically beneficial medicines that currently play main roles in the cure of human diseases [5, 32]. In this work, the extracts of *L. inermis* leaves have been determined for their antibacterial properties for controlling some Gram-positive bacteria. The results of the present study indicate different concentrations of henna extract exhibited different inhibition zones against tested bacteria isolates, Table 1.

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 [29, 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 have the ability to 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 *Staphlococcus 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 has noticed that *Staphylococcus aureus* is more susceptible than the other multi-drug resistant bacteria, to the employed plant extracts and antibiotics [32, 36].

These findings are in agreement with our data where the antibacterial activity of the phytoconstituents of *L. inermis* were 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 of this observation might be due to the variety of the antimicrobial compounds that have been isolated from the ethanolic extract [16, 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 to that achieved from other studies [38].

As demonstrated in Table 1, in *Streptococcus pneumoniae and S. agalactiae,* there was 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 [35]. By the way, Aljamali indicated that this variation in antimicrobial effects could be because of the phytochemical dissimilarities and bacterial strains differences [30, 39].

Antibacterial sensitivity testing was carried out using Tetracycline, Cefotaxime, Tobramycin and Amicacin, 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 literatures [14, 40-42].

5. 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 clearly proves the significance of plant extracts in the control of resistant microorganisms, which is becoming a hazard to human health.

Authors' Contributions: Conception and design; SM, Development of methodology; CS, Acquisition of data; SM Analysis and interpretation of data; SM, Writing, review and/or revision of the manuscript; AA, Administrative, technical, or material support; CS, Study supervision, AA. All authors read and approved the final manuscript.

Conflict of Interest: The author declares no conflicts of interest.

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