

Phytochemical Screening And Antibacterial Activity Of Yemeni Henna (*Lawsonia Inermis*) Against Some Bacterial Pathogens

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Abstract: Phytochemical screening and antibacterial activity of aqueous, methanol extracts of Yemeni henna (*Lawsonia inermis*) leaves were tested against three bacterial species including (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) using agar diffusion and minimum inhibitory concentration (MIC) as a determination method. Preliminary phytochemical screening revealed the presence of Alkaloids, Quinones, Glycosides, Tannins and saponins. The methanolic extract displayed a potential antibacterial activity against all the bacterial species, than the aqueous extract. The maximum activity was observed in methanolic extract against *Staphylococcus aureus* at inhibition zone of about (27 ± 1 mm) and minimum activity was observed in aqueous extract against *Escherichia coli* at inhibition zone of about (8.6 ± 1.2). MIC values for all the existing extracts at a concentration of 2.5mg/ml at *Staphylococcus aureus* and 10 mg/ml at *Pseudomonas aeruginosa*.

Key words: *Lawsonia inermis*, Pathogenic bacteria, Extract, Antimicrobial activity, MIC.

I. Introduction

Plants are rich in a wide variety of secondary metabolites polyphenols, such as tannins, terpenoids, alkaloids, and flavonoids, which have been demonstrated to have in vitro antimicrobial properties (1-3).

The *Lawsonia inermis* Linn belongs to the family *Lythraceae* and it is widely grown in various tropical regions in Asia, America and Africa. In Arabic, the word "henna" refers to *L. inermis* (4) and it has medicinal properties (5, 6).

Henna (*Lawsonia inermis*) plant is naturalized in Yemen and is specially growing in Taiz, Tihama, Lahj, Aden and Hadramot. It is an evergreen shrub with glabrous much branched with grayish-brown bark leaves are opposite, sub-sessile, elliptic or broadly lanceolate, entire, acute or obtuse, 2-3 long, 1-2 cm wide and dark green from its leaves obtain dark orange dye. Flowers are numerous, small, white or rose coloured and fragrant.

Henna is known to be used as a cosmetic agent for dyeing hair, nails and skin [7]. Henna contains Lawsone in about 0.5 to 1.5% of its ingredients. Henna paste is used for the skin, where the lawsone penetrates into the outermost layer of the skin and makes a red-brown stain (8).

Plants are rich in a wide variety of secondary metabolites, such as resins, tannins, terpenoids, alkaloids, flavonoids, pesticides and other pharmacological compounds it has demonstrated *in-vitro* antimicrobial (9).

The antimicrobial (10, 11) and fungicidal (12) effect of henna has long been known in previous studies (2). In this present study we investigated the effect of local Yemeni henna on three bacterial species.

II. Materials And Methods

The plant materials:

The plant materials were collected from Al-Fiuosh area at Lahj governorate, Yemen, especially in the dryness seasons and identified by the professor Dr. Al-Gifri.A, at the Biology Department, Faculty of Education/Aden, University of Aden. The plant leaves have been washed thoroughly 2-3 times under running tap water and then sterile distilled water. These leaves were dried at room temperature for two weeks in an open air protected from direct exposure to sunlight and were subsequently ground to a powder. The dried powder was stored in an air-tight bottle at 28°C for further extraction.

Extraction:

Crude extracts of each plant were obtained using two different solvents: aqueous solvent (aqueous extract) and organic solvent (methanolic extract). The methanolic extraction was carried out by soaking 30 g powder in 300 ml methanol (70%) for 48 hours at room temperature. The extract was filtered through double layered muslin cloth and then filtered through Whatman No.1 filter paper. The aqueous extraction was carried out by suspending 30g powder in 300ml sterile water, soaking for two days (48 h) after

that the extract filtered through double layered muslin cloth and then filtrated through Whatman NO 1 filter paper .

The methanolic and aqueous extract were concentrated to dryness in an oven at 45°C in order to remove the excess solvent. The extract was kept in sterile bottle under refrigerated condition (4°C) until use (13) . The dry weight of the extract was obtained by allowing the solvent to evaporate and used to determine concentration in mg/ml (14) . The extracts were dissolved in Dimethyl Sulfoxide (DMSO) to give a concentration of 100mg/ml and these were kept in a refrigerator till further use.

Phytochemical analysis:

The extracts of *Lawsonia inermis* prepared in the present study were screened for phytochemicals including carbohydrates, cardioglycosides, alkaloids, flavonoids, saponins and quinones by phytochemical analysis as below [15, 16).

Isolation Of Microorganisms:

The microbial specimens were isolated from patients suffering from different clinical conditions like (wound infection, urinary and tract infection) and included the following organisms: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and candida albicans. The processes of bacterial culturing and the identification of the bacterial and fungal specimens were done at the bacteriological laboratory of Ibn khloodon Hospital, Lahj Governorate, Yemen.

Antimicrobial Activity Assay

The Antibacterial and the antifungal activity of the two extracts of henna were individually tested against studied microorganisms. The extract were assessed using well Agar diffusion test.[17] Mullar Hinton agar and PD agar (Himedia, india) was used with different diluted extract concentrations (50 µl, 100 µl and 150 µl). The DMSO solvent was served as a negative control and the Amikacin (30 µg) (Himedia, india) for bacteria and nystatin for fungi as a positive control. The Antimicrobial activity was measured using well diffusion method according to the National Committee for Clinical Laboratory Standard.[18] All tests were performed in triplicate.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by the micro-dilution method using serially diluted (2-fold) of the plant extracts according to the [18]. A final concentration from 10 to 0.625 mg/ml was used for plant extract. The bacteria inoculation was adjusted to contain approximately 10⁵ CFU/ml. The test plates were incubated at 37 °C for 18 h.

III. Result And Discussion

Phytochemical screening:

phytochemical analysis of the methanol and aqueous extracts of *Lawsonia inermis* leaves were carried out to determine the presence of phytochemicals like alkaloids, quinones, tannins, flavonoids, saponins and glycosides (Table 1). The result showed the presence of alkaloids, glycosides and tannins in methanolic extract and alkaloids, glycosides, tannins, quinones and saponins in aqueous extract. We did not found a presence of flavonoids in both extract.

The various phytochemical compounds detected from *Lawsonia inermis* were used in medicine and have antibacterial and antifungal significance (19, 11, 8, 20).

Antibacterial activity:

The results of antibacterial activity of the *Lawsonia inermis* showed that the methanolic extracts are effective against all of the tested bacteria, than the aqueous extract. Table (2) and figure no.1 shows diameter zones of inhibition of the bacterial growth at different concentration of the leaves methanolic and aqueous extract.

The highest activity were demonstrated by the *Lawsonia inermis* against *staphylococcus aureus* and the lowest activity against *E. coli*, the respective diameter zones of inhibition were 14.3 ± 0.7, 26.6 ± 1.2, 27 ± 1 and 6.5 ± 0.5, 8.3 ± 0.6, 10 ± 1.1 mm respectively by methanolic extract. The aqueous extract was effective against *staphylococcus aureus* than the other two, where the diameter zones of inhibition were 6.3 ± 0.6, 13.2 ± 0.6 and 15 ± 1 mm.

This observation could be reasoned to the variety of the antimicrobial compounds that have been isolated from the methanolic extract (21).

In this study, the methanolic extract exhibited higher antibacterial activity against *staphylococcus aureus* compared to the Amikacin (30 µg) antibiotic.

According to the study of Papageorgiou et al. [22], phytochemical constituents of *Lawsonia inermis* exhibit antimicrobial activity only against gram positive bacteria while ineffective for gram negative bacteria. Other studies have found that *Lawsonia inermis* had antimicrobial activity against both gram positive and gram negative bacteria (20, 23, 2, 24) . Dry leaves and fresh leaves of *Lawsonia inermis* demonstrated high against *pseudomonas aerogenes* (5).

The antimicrobial activity of the methanolic extract may be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive (25). A previous study on Yemeni henna showed that alcoholic extract leaves had an *in-vitro* antibacterial activity except water extract against the tested bacterial strains (19).

In this study the MIC is expressed as the lowest concentration of the extract which gave an inhibition against the bacteria. The MIC values of methanolic extract against *staphylococcus aureus* were at 2.5 mg / ml and *Pseudomonas aerogenes* at 10 mg/l Table (3). The MIC values of this study was less than the MIC values reported by Al-kurashy et al. [26]. They found MIC values in the range of 8–64 mg/ml for aqueous extract and 32–64 mg/ml for alcoholic extract of *Lawsonia inermis* against *E. coli*, *S. aureus*, *P. aureginosa* and *E. faecalis*. In conclusion, the present study encourages the medicinal use of *Lawsonia inermis* and it also indicates that the use of a great significance as a substitute antimicrobial agent in therapeutics.

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Table (1): Phytochemical analysis of different extracts of *Lawsonia inermis*

Phytochemicals	Test/reagents	<i>lawsonia inermis</i>	
		Aqueous	Methanolic
Alkaloids	Mayer's test	+	+
Quinones	Sodium Hydroxide	+	–
Flavonoids	Lead Acetate Test	–	–
Saponins	Foam test	+	–
Glycosides	Fehling's solution (A, B)	+	+
Tannins	Ferric chloride test	+	+

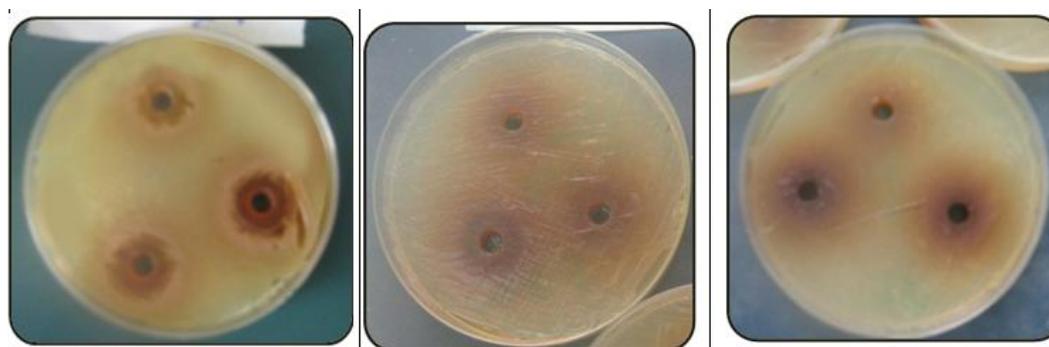
Table (2): Antibacterial activity of *Lawsonia inermis* methanolic and aqueous extracts against some bacterial Pathogens.

Microorganisms	Zone of inhibition (mm) (Mean ± SD)						Amikacin (30 µg)
	Methanolic extract			Aqueous Extract			
	50 µl	100 µl	150 µl	50 µl	100 µl	150 µl	
<i>Staphylococcus Aureus</i>	14.3 ± 0.7	26.6 ± 1.2	27 ± 1	6.3 ± 0.6	13.2 ± 0.6	15 ± 1	17
<i>Pseudomonas aerogenes</i>	8.2 ± 0.8	11 ± 1	15 ± 1	7 ± 1	8.6 ± 1.1	11 ± 1	15
<i>E. coli</i>	6.5 ± 0.5	8.3 ± 0.6	10.7 ± 1.1	6	6	8.6 ± 1.2	16

Table (3): The Minimum inhibitory concentration (MIC) of *Lawsonia inermis* methanolic and aqueous extracts against some bacterial Pathogens.

Minimum inhibitory concentration (MIC) in mg/l		
Microorganisms	Methanolic extract	Aqueous extract
<i>Staphylococcus aureus</i>	2.5 mg/ml	10 mg/ml
<i>Pseudomonas aeruginosa</i>	10 mg/ml	Nd*
<i>Escherichia coli</i>	nd	Nd*

Nd*: no detect



Staphylococcus aureus

Pseudomonas aeruginosa

E. coli

Fig.No.1. Zone of inhibition (in mm) of methanolic extract of *Lawsonia inermis* leaves against pathogen bacteria