

***In vitro* Antibacterial activity and Flavonoid contents of *Lawsonia inermis* (Henna)**

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ABSTRACT: *Lawsonia inermis* known as henna is a woody and flowering plant found in north Africa and south west asia. Flavonoids which were reported as having many pharmacological activities, antimicrobial, antioxidant, cytotoxic, chemo-prevention activities and they possess strong anti-proliferative effects related to inhibition of cell cycle progression and apoptosis induction. On the basis of this *Lawsonia inermis* (L.) syn. *Lawsonia alba* Henna (family Lythraceae) was selected and it is having the major composition of flavonoids and the antibacterial activity of methanolic extract of *Lawsonia inermis* was investigated by agar well diffusion method. The bacteria used for antibacterial study were *Staphylococcus aureus* (MTCC 087), *Escherichia coli* (MTCC 729), *Klebsiella pneumoniae* (MTCC 432), *Pseudomonas aeruginosa* (MTCC 1688) & *Proteus mirabilis* (MTCC 425). According to our results in the lowest tested concentration of 62.5µg/ml and 125µg/ml 7.2% of the plant extract were active, 5% active in the concentration of 250µg/ml, 75.7% active in the concentration of 500µg/ml and 92.8% active at the concentration of 1000µg/ml in a dose dependent manner.

Keywords: *Lawsonia inermis*, Antibacterial activity, Agar well diffusion, Lythraceae, methanol extract.

INTRODUCTION

Plants are used as medicines since time immemorial. India has a rich heritage of using medicinal plants in traditional medicines such as Ayurveda, Siddha and Unani besides folklore practices [2]. *Lawsonia inermis* syn. *Lawsonia alba* (Henna) is a sole species in the genus in the family Lythraceae [8]. Henna has been found to exhibit Antibacterial, Antifungal and Dermatological properties. It is useful in coloring of skin, scalp and nails etc. Henna has also shown anti-diarrhoeal, diuretic, emmanagogue and abortifacient prophetically and is found to be practically non-toxic [4]. Alcoholic extract of shade dried leaves of *Lawsonia inermis* intraperitoneally injected to rats showed anti-inflammatory activity comparable to that of hydrocortisone [7]. Chloroform extract of leaves exhibit promising antibacterial activity against *Shigella* and *vibrio cholera* [1] Astringent properties [3]. No antifungal activity was detected for henna aqueous solutions [5]. Out of 24 plant products evaluated their

antibacterial activity against six potato pathogenic bacteria, 18 products exhibited antibacterial activity. Gallic acid as found to be the most potent antibacterial agent followed by lawsone [6]. Many plants derived from nature possess antimicrobial and insecticidal activities. The interest in these plants is increasing because of finding safer microbicides in combination with the need of preventing environmental degradation. For centuries preparations containing flavonoids as the principal physiologically active constituents have been used to treat Human Diseases. Increasingly, this class of natural products is becoming the subject of anti-infective research and many groups have isolated and identified the structures of flavonoids possessing antimicrobial and cytotoxic activities [9].

Henna has been used to treat skin infections such as tinea and it is known to have antibacterial properties which have been attributed to naphthoquinones, including lawsone [14]

Reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to their inter and intra assay variation in susceptibility testing^[10]. However, several high quality investigations have examined the relationship between flavonoid structure and antibacterial activity and these are in close agreement. Most of the medicinal plants have identified and used for treatment of human diseases are well documented^[11].

MATERIALS AND METHODS

Maintenance of isolates

A standard MTCC strains belonging to five different species of gram positive cocci and gram negative bacilli were maintained on Brain Heart Infusion Agar (BHA- Himedia)

Preparation of crude extract

The powdered plant materials (10gms) were extracted with 100ml of methanol for 1hr on an ultrasonic bath. The extract was filtered the filtrate was evaporated in vacuum at 45°C and then lyophilized. The extracts were prepared according to the polarity^[12].

Preliminary phytochemical screening

The preliminary phytochemical screening of *Lawsonia inermis* was carried out for the detection of various Phyto-constituents using standard procedure^[13] The following solvents were used for the study, petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. The methanolic extract was found to contain more flavonoids. The preliminary phytochemical screening of methanolic extract reveals the presence of alkaloids, flavonoids, tannins and quinones.

Chemicals

Ampicillin (10 µg/ml) discs, Molten Mueller Hinton(MH) Agar and Nutrient agar medium were obtained from Hi-media laboratories, Mumbai. All other chemicals were of analytical grade and obtained locally.

Test microorganisms

The bacteria used for antibacterial study were *Staphylococcus aureus* (MTCC 087), *Escherichia coli* (MTCC 729), *Klebsiella pneumoniae* (MTCC 432), *Pseudomonas aeruginosa* (MTCC 1688) & *Proteus mirabilis* (MTCC 425).

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination Each plant extract (0.5 ml of

1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% Aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Antibacterial study (plate hole diffusion method)

The plate hole diffusion assay was used to determine the growth inhibition of bacteria by plant extracts. Bacteria were maintained at 4°C on nutrient agar plates before use. Nutrient agar was prepared and 25ml of each was poured in to sterile universals. The universals with the broth were inoculated with different species of bacteria and incubated at 37°C overnight. A total of 25ml of molten Muller Hinton (MH) agar (Himedia) held at 40°C was poured in to sterile universals maintained at 40°C in a water bath. Each universal was inoculated with 0.2ml of different bacterial species mixed well, transferred in to sterile Petri dishes and allowed to set. Using a sterile cork-borer 6mm diameter, four holes per plate were made in to the set agar containing the bacterial culture. A total of 0.2ml of plant extracts were poured in to the wells and one containing distilled water, the plates were kept in incubator overnight and the zone diameter was then recorded if greater than 6mm.

Screening of Antibacterial activity (*Lawsonia inermis*)

The methanolic extract of *Lawsonia inermis* leaves for antibacterial screening. The results of the total flavonoid constituent and study was 25.05 ± 0.18 respectively (table 1). The Five bacteria were used for antibacterial screening. Various concentrations of methanolic extract were used (1000µg/ml, 500µg/ml, 250µg/ml, and 62.5µg/ml) to test the antibacterial activity. From the results of antibacterial screening, 7.2% of methanolic extract were active in the lowest tested concentration of 62.5µg/ml, 5% active in a concentration of 250µg/ml, 75.7% active in a concentration of 500µg/ml, and 92.8% active in a concentration of 1000µg/ml. Ampicillin (10µg) was used as standard drug.

Preliminary phytochemical screening

The preliminary phytochemical screening reveals the presence of Flavonoids Alkaloids, Tannins and quinones. The results were shown in table 1&2.

Flavonoid contents in Lawsonia inermis**Table-1**

PLANT NAME	Flavonoids (mg/g)
<i>Lawsonia inermis</i>	25.05±0.18

Qualitative test**Table-2**

	Lawsonia inermis
Alkaloids	+
Quinones	+
Tannins	+
Flavonoids	+

+ Present ; - Absent

Table 3: Antibacterial study of methanolic extract of *Lawsonia inermis*

Bacteria	Zone diameter (mm)*					
	1000µg/ml	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	Ampicillin 10µg/ml
<i>Staphylococcus aureus</i> (MTCC 087)	24	18	13	9	9	28
<i>Escherichia coli</i> (MTCC 729)	22	20	12	8	0	24
<i>Klebsiella pneumoniae</i> (MTCC 432)	18	14	9	6	0	24
<i>Pseudomonas aeruginosa</i> (MTCC 1688)	17	14	12	6	0	22
<i>Proteus mirabilis</i> (MTCC 425).	23	18	10	6	0	24

DISCUSSION

The present study was conducted to study the *in vitro* antibacterial activity of *Lawsonia inermis* used by Indian peoples to show that therapeutic properties. The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent. Five bacteria were used for antibacterial studies.

Medicinal plants are being used by large proportion of Indian population. The reasons for this include a) True

improvement of diseases conditions after herbal treatment b) Harmful side effects and high cost of the other forms of treatment. In the present study, the results were encouraging, as the *Lawsonia inermis* appeared to contain substances that had antimicrobial properties because of, the methanolic extract of *lawsonia inermis* leaves were active against five different bacteria's. Five concentrations of the extract were used (1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml

and 62.5µg/ml). It is estimated that if an inhibition is obtained by 250µg/ml-1000µg/ml) of test solution, the extract can be considered worthy for further investigations. Plants showing significant activity may

be due to the presence of alkaloids, flavonoids, tannins and quinones. Among the various microorganisms, the methanolic extract of *lawsonia inermis* was more active against *Staphylococcus aureus*.

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