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A Study of Extract Optimization and Antibacterial Properties of *Lawsonia inermis*

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Abstract : The antibacterial properties of "*Lawsonia inermis*" commonly known as "Henna or Mehandi" tested against bacterial pathogens (*S. aureus, P. aeruginosa and E. coli*). Agar diffusion susceptibility test revealed inhibition zone of Henna sample. The Henna leaves were exhibiting best result followed by fruits, bark root and stems. The solvents were used ethanol, methanol, ethyl acetate and hot water, compare to all, ethanolic extract and ethyl acetate extract were showing best result against one gram positive culture *Staphylococcus aureus* (MTCC 2940) and two gram negative cultures *Pseudomonas aeruginosa* (MTCC 2453) and *E. coli* (MTCC 739). The MIC value was determined by using broth dilution methods. Ethanolic extract of Henna was subjected to get the MIC against test organisms and it was found to be 1.45 mg/ml for *E. coli* and *Pseudomonas aeruginosa*.

***Keywords*:** Antibacterial properties, ethanolic and ethyl acetate plant extract, MIC, zone of inhibition.

Introduction:

Medicinal plant occupied an important position in the socio, cultural and spiritual arena of rural people in many parts of worlds. World Health Organization [1] estimated that 80% of the population of developing country rely on traditional medicines mostly plant drugs. The microorganisms have developed resistance to many antibiotics because of continuously uses of antimicrobial drug that create a problem in treatment of infectious diseases [2]. Herbal plant such as Henna (*Lawsonia inermis*) contains high amount of flavanol and phenolic acid [3], also known as antioxidants to help to reduce free radicals by many ways from medication to food additives. There is a widespread believed that compare to synthetic medicines green medicines are healthier and safer [4]. Antibacterial properties of various parts like root, stem, leaves and flowers have been well documented for some of the medicinal plants for the past two decades [5]. *Lawsonia inermis* is a flowering plant, the sole species in the genus *Lawsonia* in the family lythraceae. It is native to tropical and subtropical regions of Africa, Southern Asia and Northern Australia in semiarid zones [6]. *Lawsonia inermis* is a shrub or small tree (2-6 m height). It's traditional name is Mehndi and applied to hair, feet and hands. Henna symbolizes fertility and also it is in use because of it's cooling effect. Henna's leaves, flowers, seeds and stems, bark and roots are used in traditional medicines to treat a variety of ailments as rheumatoid arthiritis, headache, ulcers, diarrheoa, leprosy and fever etc.

Present study is carried out to check the antimicrobial activity of *Lawsonia inermis* against 3 bacterial pathogens. The samples used were plant leaves, seeds, roots and stems in different solvents like Ethanol, Methanol, Ethyl acetate and Hot water.

Materials and Methods:

Collection of Plant:

The *Lawsonia inermis* leaves, seeds, roots, barks and stems were collected from local area in Gomti Nagar, Lucknow.

Preparation of Plant Extract:

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant. .*Lawsonia inermis* leaves, seeds, roots and stems were washed with distilled water and kept in incubator at 37°C for 3-4 days and grinded into fine powder. Now plant material was dissolved in 70% ethanol and 80% methanol, ethyl acetate and hot water (1:10), 1 g sample should be dissolved in 10 ml of solvent. Mixtures were kept in the dark for 3 days at room temperature in sterilized beakers wrapped with aluminum foil to avoid evaporation and exposure to sunlight was avoided. After 3 days, mixtures were filtered through Whatman no.1 filter paper and kept it in incubator at 37°C till all solvents had completely evaporated from mixtures. Now all mixtures were dissolved in DMSO (Dimethyl sulfoxide).

Tested Microorganisms:

Bacterial cultures were obtained from IMTECH, Chandigarh. Subcultures were maintained by MRD LifeSciences, Lucknow. One gram positive culture- *Staphylococcus aureus* (MTCC 2940) and two gram negative cultures- *Pseudomonas aeruginosa* (MTCC 2453) and *E. coli* (MTCC 739) were used.

Antibiogram Analysis:

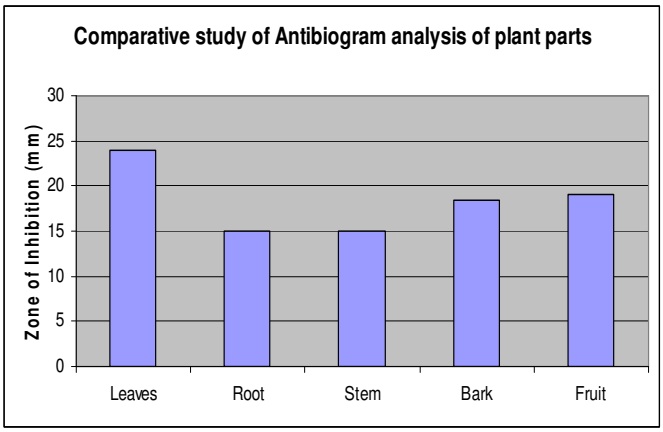
The antimicrobial activity of *Lawsonia inermis* was evaluated against bacterial strains in Ethanolic, Methanolic, Ethyl acetate and Hot water by using agar well diffusion method [7]. Nutrient agar plates were prepared for all extracts, 50µl inoculum of each selected bacterium was uniformly spreaded on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. The equal volume (50µl) of antibiotic (tetracycline),

distilled water and plant extract were poured into the wells. The plates were incubated at 37°C for 24 hrs.

Determination of minimum inhibitory concentration (MIC) of ethanolic, methanolic, ethyl acetate and hot water extract:

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation at 370C in shaker incubator [8][9]. MIC of all samples were determined by broth dilution method. A two-fold serial dilution of the methanolic, ethanolic, ethyl acetate and hot water extracts were prepared and optical density was measured at 600 nm [10].

Results: Antibiogram of Leaves Extract of Lawsonia inermis



Graph showed that leaves were having maximum antibacterial activity followed by fruits, bark, stem and root.

Table 1: **Methanolic Extract of Leaves.**

Pathogens	Zone of inhibition (mm.)		
	Tetracycline	Plant extract	Distilled water
Staphylococcus aureus	15	22	0
Pseudomonas aeruginosa	15	21	0
E. coli	23	19	0

Table showed that *S. aureus* was having maximum zone of inhibition compare to *E. coli* and *P. aeruginosa*, which was higher than tetracycline.

Table 2: **Ethanolic Extract of Leaves.**

Pathogens	Zone of inhibition (mm.)		
	Tetracycline	Plant extract	Distilled water
Staphylococcus aureus	15	24	0
Pseudomonas aeruginosa	15	23	0
E. coli	23	20	0

Table showed that *S. aureus* was having maximum zone of inhibition compare to *E. coli* and *P. aeruginosa*, which was higher than tetracycline.

Table 3: **Ethyl Acetate Extract of Leaves.**

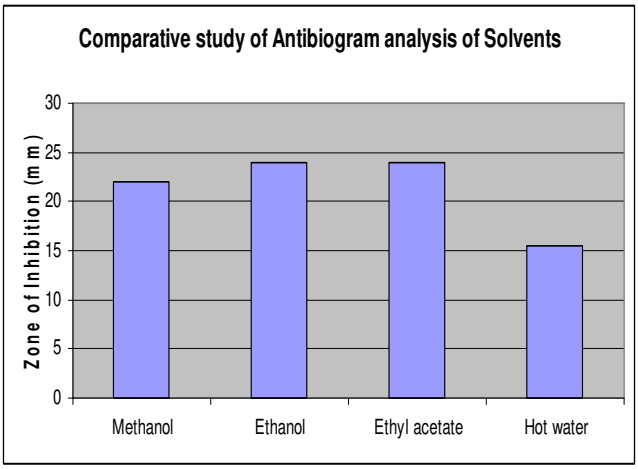
Pathogens	Zone of inhibition (mm.)		
	Tetracycline	Plant extract	Distilled water
Staphylococcus aureus	15	21	0
Pseudomonas aeruginosa	15	24	0
E. coli	23	24	0

Table showed that *E. coli* and *P. aeruginosa* were having maximum zone of inhibition compare to *S. aureus* which was higher than tetracycline.

Table 4: **Hot Water Extract of Leaves.**

Pathogens	Zone of inhibition (mm.)		
	Tetracycline	Plant extract	Distilled water
Staphylococcus aureus	15	14	0
Pseudomonas aeruginosa	15	14	0
E. coli	23	15.5	0

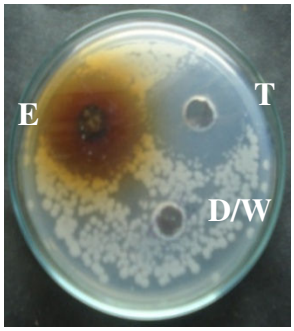
Table showed that *E. coli* was having maximum zone of inhibition compare to *S. aureus* and *P. aeruginosa* which was higher than tetracycline.



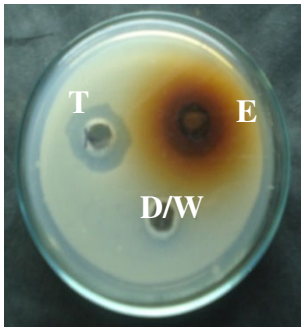
Graph showed that Ethanolic leaves and Ethyl acetate leaves extracts were having maximum antibacterial activity compare to Methanol and Hot water.

Figure 1: **Methanolic Extract of Leaves.**

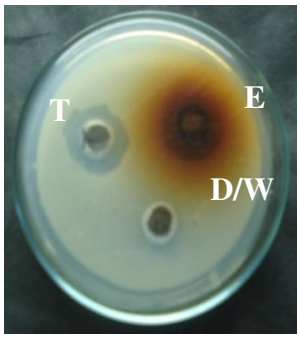
Zone of inhibition was higher in case of *S. aureus* compare to *E. coli* and *P. aeruginosa*



E. coli

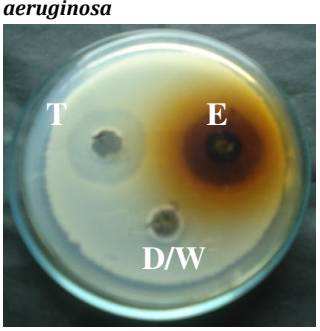


P. aeruginosa

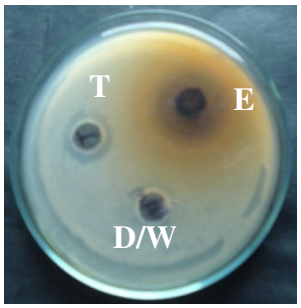


S. aureus

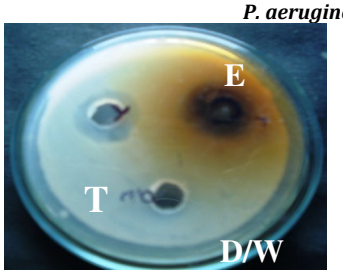
***Figure 2*: Ethanolic Extract of Leaves.**
Zone of inhibition was higher in case of *E. coli* than *S. aureus* and *P. aeruginosa*



E. coli

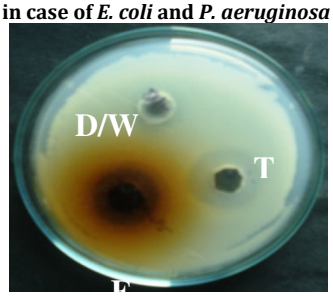


P. aeruginosa

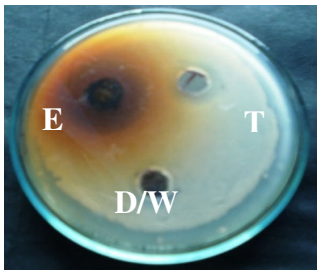


S. aureus

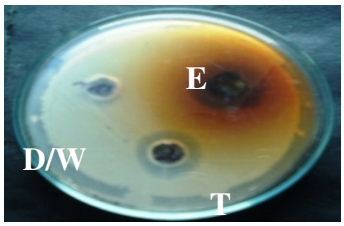
***Figure 3:* Ethyl Acetate Extract of Leaves.**
The leaves extract in ethyl acetate showed higher zone of inhibition in case of *E. coli* and *P. aeruginosa* than *S. aureus*



E. coli

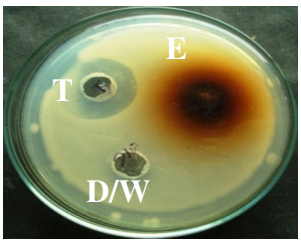


P. aeruginosa

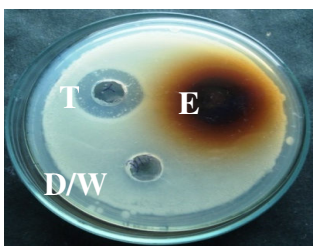


S. aureus

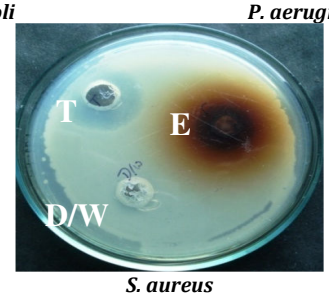
***Figure 4:* Hot Water Extract of Leaves.**
Zone of inhibition was higher in case of E. coli than S. aureus and P. aeruginosa



E. coli



P. aeruginosa



S. aureus

***Table 5:* MIC of Ethanolic Extracts Leaves of *Lawsonia inermis* against Bacterial Pathogens.**

Test tube	Conc. of Ethanolic extracts (mg/ml)	O.D. against *E. coli* (600nm)	O.D. against *Pseudomonas aeruginosa* (600nm)
1	71.248	0.00	0.01
2	10.204	0.05	0.18
3	**1.457**	**0.01**	**0.10**
4	0.208	0.30	0.20
5	0.029	0.35	0.29
6	0.004	0.40	0.34

Chart showed that least concentration of ethanolic extracts leaves was 1.45 mg against *E. coli* and *P. aeruginosa*

***Table 6:* MIC of Ethyl Acetate Extracts Leaves of *Lawsonia inermis* against Bacterial Pathogens.**

Test tube	Conc. of Ethyl acetate extracts (mg/ml)	O.D. against *E. coli* (600nm)	O.D. against *Pseudomonas aeruginosa* (600nm)
1	71.248	0.01	0.05
2	10.204	0.05	0.71
3	**1.457**	**0.01**	**0.20**
4	0.208	0.18	0.48
5	0.029	0.22	0.49
6	0.004	0.23	0.53

Chart showed that least concentration of ethyl acetate extracts leaves was 1.45 mg against *E. coli* and *P. aeruginosa*

Discussion:

Present time the emergence of multi-drug resistance in human and animal pathogenic microbes as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drug of plant origin. The antimicrobial activity of leaves extracts of *L. inermis* (Henna) and their potency was quantified by the zone of inhibition measurement **[11].** All plant parts were having antibacterial activity but compare to all, leaves were having maximum antibacterial activity than fruits, bark, root and stems. The solvents used were Ethanol, Methanol, Ethyl acetate and Hot water and after Antibiogram analysis it was observed that Ethanol and Ethyl acetate were having maximum antibacterial activity compare to Methanol and Hot water extract.

The Antibiogram was done against 3 pathogens which were *E. coli*, *S. aureus* and *P. aeruginosa* and after observation it was found that ethanolic and methanolic leaves extracts were having maximum zone of inhibition against *S. aureus* which were 24 mm and 22 mm in diameter and also the ethyl acetate extracts were having maximum zone of inhibition (24 mm) against *P. aeruginosa* and *E. coli*, which were higher than the result obtained by [12], while the hot water extract was having minimum zone of inhibition against all the cultures.

MIC is the least concentration of antibiotics which inhibit the growth of other microbes.

The MIC value of ethanolic extracts leaves of *Lawsonia inermis* and also Ethyl acetate extracts leaves of *Lawsonia inermis* obtained 1.45 mg against *P. aeruginosa* and *E. coli.* which was more compare to previous work **[13].**

Conclusion:

Henna (*Lawsonia inermis*) has been used since earliest time as a medicines, preservative and cosmetics. It has long been recommended in traditional eastern medicines as an astringent and purgative. From the results of antibacterial screening of four solvents i.e. Ethanol,

Methanol, Ethyl acetate and Hot water used in this study, the solvents ethanol and ethyl acetate were showing best result and among the parts of plant the leaves sample were having maximum activity compare to fruits, bark, root and stems. The inability of extracts of selected plants to demonstrate any visible activity against some bacteria may probably due to low concentration of extracts.

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