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Evaluation of Antimicrobial Activity and Phytochemical Analysis of Leaves and Stems of *Lawsonia Inermis*

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ABSTRACT

In this present study, the crude extracts from leaves and stems of *Lawsonia inermis* (Henna) in different solvent, were subjected for antimicrobial analysis against bacterial pathogens *(P. aeruginosa, S. aureus* and *E. coli*) and fungal pathogens which are *A. niger, C. albicans, T. rubrum* and *M. canis* and phytochemical analysis. The dry crude henna extract was tested for its antimicrobial activities using "agar well diffusion antibiotic susceptibility technique". The henna stems were showing best result as compared to leaves. The solvents used were ethanol, methanol, ethyl acetate and hot water, compare to all, ethanolic extract and ethyl acetate extract were showing best result against *S. aureus* and *E. coli*. The MIC value was determined by using broth dilution method. MIC values were obtained 0.02 mg/ml for ethanolic extract and 0.38 mg/ml for ethyl acetate extract against *S. aureus* and 0.38 mg/ml for ethanolic and ethyl acetate extract against *M. canis*. The Phytochemical study showed that Cardio glycosides, terpenoids and phenols compounds were present in plant samples.

Key words: Antimicrobial activities; Ethanolic and Ethyl acetate plant extracts; MIC and Phytochemical analysis.

INTRODUCTION

The antibiotic was coined by Selman Waksman in 1942. Antibiotics are generally used against bacteria. Antibiotics are currently in use to treat a variety of infectious human disease but there are so many cases found in that side-effects were seen so natural plant can be used as a medicine with no side effects. A plant extract is a mixture of phytochemical. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids which have been demonstrated to have in vitro antimicrobial properties. Antimicrobial is the name for a chemical that either kills or prevents the growth of microbes such as bacteria, fungi or protozoa. Antibiotic is a compound or substance which inhibits or slow down the growth of bacteria. Antifungals are drugs that are used to treat fungal infections. Herbal medicine is a complementary therapy that uses plants or plant extract to treat illness. Herbal medicine is also called botanical medicine or phytomedicine refers to using a plant seeds, roots, leaves, barks or flowers for medicinal purpose. Henna is native to tropical and subtropical regions of Africa and Southern Asia. It is tall shrub or small tree 2-6 meters height. It is multi branched with spine branchlets. The used parts of henna are leaves, stems, roots, flowers, fruits and barks for medicinal and manufacturing different products. Henna is world wide known for the beautiful colouring dye. Binomial name is *Lawsonia inermis* and common name is Jamaica mignonette **(Gupta AK, 2003)**. The use of medicinal plant as a source of relief from illness **(Thomson *et al*., 1978)**.70% of the world population is dependent on the traditional medicines for primary healthcare. India is known for its rich diversity of medicinal plants and hence called botanical garden of the world **(Vedavathy *et al*., 1997).** Isolation of antimicrobial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world **(Benjar, 2004)**. Wound may be defined as less or breaking of cellular and anatomic or functional continuity of living tissues. In traditional systems of medicine, various plants have been used to promote wound healing .Henna has been used for astringent, antihemorrhagic, intestinal anti-neoplastic, cardio-inhibitory, hypotensive and sedative effects and used as a folk remedy against amoebiasis, headache, jaundice and leprosy **(Sastri, 1952**). Many of the plants used for dye extraction are classified as medicinal and some of these have recently been shown to possess antimicrobial activity. The therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms **(Rawat, 2003)**. The present study is carried out by evaluation of antimicrobial activity of leaves and stems of *Lawsonia inermis* against bacterial pathogens *(P. aeruginosa, S. aureus and E. coli*) and fungal pathogens (*A. niger, T. rubrum, M. canis, C. albicans*) and also phytochemical analysis which are responsible for antimicrobial activity.

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MATERIALS AND METHOD

The Sample *Lawsonia inermis* was collected from Vibhav Khand (Gomti Nagar) Lucknow. Used parts of sample were leaves and stems.

Bacterial and Fungal cultures were obtained from IMTECH, Chandigarh. Subcultures were maintained by MRD LifeSciences, Lucknow. Bacterial cultures used were- *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* and Fungal cultures used were- *A. niger, C. albicans, M. canis* and *T. rubrum*.

Four solvents were used in this experiment- 80% methanol**,** 70% ethanol**,** 100% ethyl acetate and hot water.

Sample should be washed with distilled water air dried in oven and grind properly all the leaves and stems to get powder form. Weigh 5gm of sample for each solvent. All sample kept in dark region for 2-4 days (to dissolve secondary metabolites properly). Air dried the filtrate and dissolve in double amount of Di methyl sulfoxide (DMSO). Plant extracts were ready for further work.

Antibiogram analysis method was performed to evaluate the antimicrobial properties of plant extract with the help of agar well diffusion method **(Ahmad *et al*., 2001).** 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 and observed zone of inhibition and for fungus cultures, PDA plates were prepared for all extracts, 50µl inoculum of each selected fungus was uniformly spreaded on PDA 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 room temperature for 24-48 hrs and observed zone of inhibition.

Minimum inhibitory concentration is the lowest concentration of an antimicrobial that inhibits or kills the visible growth of microorganisms. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism **(Baur *et al.*, 1966).** Prepare nutrient broth then add antibiotics in first test tube, mixed properly and transferred 1 ml in second tube and so on. In last test tube discard same amount of solution. After this step, add 20µl of the bacterial pathogen and kept at shaker incubator for overnight at 37^0C and observe result in the form of turbidity and take OD at 600 nm and for fungus cultures, prepare potato dextrose broth then add antibiotics in first test tube, mixed properly and transferred 1ml in second tube and so on. In last test tube discard same amount of solution. After this step, add 20 µl of the fungal pathogen. Incubate at room temperature for 24-48 hrs. Observe result in the form of turbidity and take OD at 600 nm.

The bacteriostatic or bacteriocidal concentration in the form of growth of bacterial culture which can be seen in the form of colonies on petriplates. Prepare NA plates. Spread 20µl of MIC tube cultures according to their arrangement. Incubate at 37^0C for overnight. Observe results in the form of bacterial colonies. Prepare PDA plates. Spread 20µl of MIC tube cultures according to their arrangement. Incubate at room temperature for 24-48 hrs. Observed result in the form of fungal colonies.

Phytochemical are the main constituents of any plant sample, which are responsible for secondary metabolites also. The other works of these phytochemical are flavouring, colours etc **(Thenmozhi.*et al*., 2010)**.

Cardio glycosides: A volume of 5ml of the plant extract was treated with 2ml of glacial acetic acid containing a drop of ferric chloride solution. Then it was underplayed with 1ml concentrated sulphuric acid. A brown ring of the interface indicates a de oxy sugar characteristic of cardio glycosides.

Carbohydrates: The extracts were treated with 5ml of Fehling's solution and kept in boiling water bath. The formations of yellow or red colours precipitate indicate the presence of reducing sugars.

Saponins: 2gm of the powdered sample was boiled in 20ml of distilled water bath and filtered. The 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously, a suitable persistent froth formed. The frothing was mixed with 3 drops of olive oil and shaken vigorously and then the formation of emulsion was observed.

Oils and fats: Press a small quantity of powder between two filter papers. Oil strain on the filter paper indicates the presence of fixed oils.

Terpenoids: A volume of 5ml of the plant extract was mixed in 2ml of chloroform and concentrated sulphuric acid was added to form a layer. A reddish brown coloration of the interface was formed to show the presence of terpenoids.

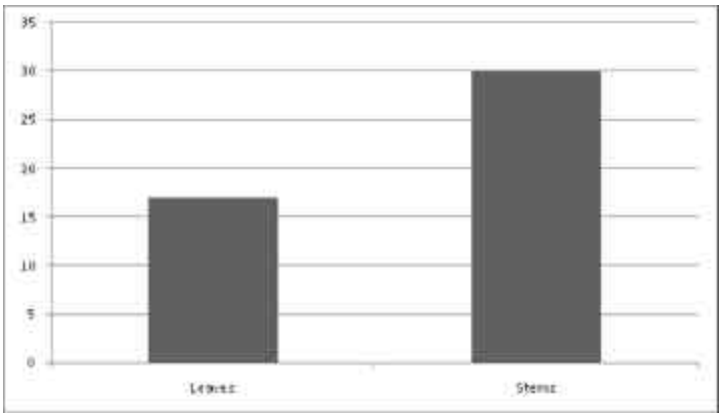
Tannins: About 0.5gm of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Phenols: A few drops of alcohol and ferric chloride solution were mixed with the plant extract. A blue green or red colour indicates the presence of phenols.

Amino acids and Proteins: Take 1ml extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. Blue colour develops indicating the presence of proteins.

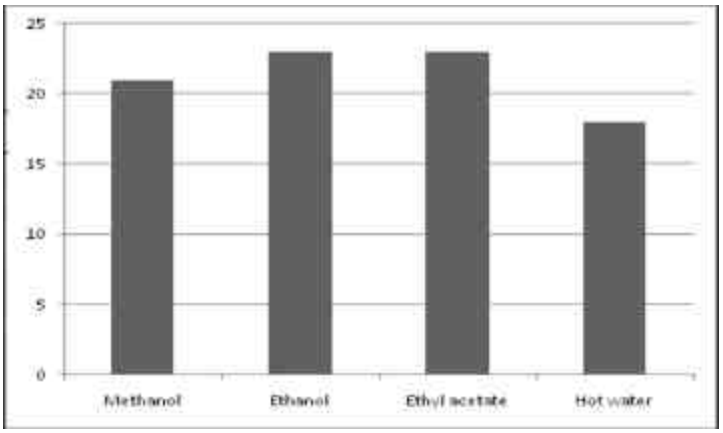
Quinones: A few drops of sodium hydroxide mixed with plant extract and shaken vigorously. A blue green red or colour indicates the presence of quinones.

RESULTS:



Graph 1: Comparative study of antibiogram analysis of plant parts

Graph 1 showed that stems were having maximum antimicrobial activity.



Graph 2: Comparative study of Antibiogram analysis of solvents

Graph 2 showed that ethyl acetate stems extract were having maximum antimicrobial activity compare to methanol, ethanol and hot water.

Antibacterial activity of henna stems:

Table 1: Ethanolic extract of henna stems

Pathogens	Zone of inhibition (mm)		
	Plant extract	Tetracycline	Distilled water
E. coli	18	20	0
P. aeruginosa	17	24	0
S. aureus	18	21	0

Table 1 showed that the maximum zone of inhibition was observed in ethanolic extract of henna stems against *E. coli.*

Table 2: Ethyl acetate extract of henna stems

Pathogens	Zone of inhibition (mm) Plant extract	Tetracycline	Distilled water
E. coli	14	21	0
P. aeruginosa	13	23	0
S. aureus	22	22	0

Table 2 showed that the maximum zone of inhibition was observed in ethyl acetate extract of henna stems against S. aureus.

Antifungal activity of henna stems:

Table 3: Ethanolic extract of henna stems

Pathogens	Zone of inhibition (mm) Plant extract	Tetracycline	Distilled water
A. niger	10	0	0
C. albicans	13	0	0
T. rubrum	14	0	0
M. canis	25	24	0

Table 3 showed that the maximum zone of inhibition was observed in ethanolic extract of henna stems against *M. canis* which was higher than tetracycline.

Table 4: Ethyl acetate extract of henna stems

Pathogens	Zone of inhibition (mm) Plant extract	Tetracycline	Distilled water
A. niger	0	0	0
C. albicans	0	0	0
T. rubrum	0	0	0
M. canis	30	29	0

Table 4 showed that the maximum zone of inhibition was observed in ethyl acetate extract of henna stems against *M. canis* which was higher than tetracycline.

MIC Results:

Table 5: MIC of ethanolic and ethyl acetate extract of henna stems against bacterial and fungal pathogens:

Test tubes	Conc. of extract mg/ml	OD of ethanolic extract against *S. aureus* (600nm)	OD of ethyl acetate extract against *aureus* (600nm)	OD of ethanolic extract against *M. S.canis*(600nm)	OD of ethyl acetate extract against *M. canis* (600nm)
1	83.33	0.01	0.32	0.69	0.69
2	13.88	0.02	0.59	0.76	0.03
3	**2.31**	**0.02**	**0.38**	0.69	0.01
4	**0.38**	0.07	0.39	**0.21**	**0.00**
5	0.06	0.21	0.44	0.20	0.00
6	0.01	0.22	0.50	0.16	0.00

Table 5 showed that MIC were obtained 0.02 mg/ml for ethanolic extract against *S. aureus*, 0.38 mg/ml for ethyl acetate extract against *S. aureus* and 0.38 mg/ml for ethanolic and ethyl acetate extract against *M. canis*.

Table 7: Phytochemical analysis

Compounds	*Lawsonia inermis* Leaves	Stems
Cardio glycosides	+	+
Carbohydrates	_	_
Terpenoids	+	+
Oils and fats	_	_
Tannins	_	_
Phenols	+	+
Saponins	_	_
Amino acids	_	_

Table 7 showed that cardio glycosides, terpenoids and phenols compounds were present and remaining carbohydrates, oils and fats, tannins, saponins and amino acids were absent.

Antibiogram analysis of bacterial and fungal pathogens:

E. coli *P. aeruginosa* *S. aureus*

Figure 1: Ethanolic extract of henna stems

Fig 1 showed that ethanolic extract of henna stems were showing maximum antibacterial activity in the form of zone of inhibition against *E. coli* and *S. aureus.*

E. coli *P. aeruginosa* *S. aureus*

Figure 2: Ethyl acetate extract of henna stems

Fig 2 showed that ethyl acetate extract of henna stems were showing maximum antibacterial activity in the form of zone of inhibition against *S. aureus*.

A. niger *C. albicans* *T. rubrum* *M. canis*

Figure 3: Ethanolic extract of henna stems

Fig 3 showed that ethanolic extract of henna stems were showing maximum antifungal activity in the form of zone of inhibition against *M. canis* compare to *A. niger, T. rubrum and C. albicans.*

A. niger *C. albicans* *T. rubrum* *M. canis*

Figure 4: Ethyl acetate extract of henna stems

Fig 4 showed that ethyl acetate extract of henna stems were showing maximum antifungal activity in the form of zone of inhibition against *M. canis* compare to *A. niger*, *T. rubrum* and *C. albicans.*

DISCUSSION

In this study the result showed that henna samples from local region of Lucknow, demonstrated antimicrobial activity against different bacterial pathogens *E. coli, P. aeruginosa* and *S. aureus* and fungal pathogens *A. niger, C. albicans, T. rubrum* and *M. canis*. Herbal medicines are valuable and readily available resources for primary health care and complementary health care system. These plants may prove to be antimicrobial activities, but more pharmacological investigations are necessary. 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 extract of *L. inermis* and their potency was quantified by the zone of inhibition measurement **(Cowan *et al* 1999).** All plant parts were having antimicrobial activity but compare to all, stems were having maximum antibacterial and antifungal activity. The solvents used were methanol, ethanol, ethyl acetate and hot water and after antibiogram analysis it was observed that ethanol and ethyl acetate were having maximum antibacterial and antifungal activity compare to methanol and hot water extract. The antibiogram was done against three bacterial pathogens and after observation it was found that ethanolic leaves extract and ethyl acetate stems extract were having maximum zone of inhibition against *E. coli* and *S. aureus* which were 22 mm and 22 mm in diameter and which were higher than the result obtained by **Sudharameshwari.K and Radhika.J. 2007.** The antibiogram was done against four fungal pathogens and after observation it was found that ethanolic stems extract and ethyl acetate stems extract were having maximum zone of inhibition against *M. canis* which were 25 mm and 30 mm in diameter. MIC is the least concentration of antibiotics which inhibit the growth of microorganisms. MIC values were obtained 0.02 mg/ml for ethanolic extract against *S. aureus*, 0.38 mg/ml for ethyl acetate extract against *S. aureus* and 0.38 mg/ml for ethanolic and ethyl acetate extract against *M. canis* **(Pandey *et al*, 2011).** The phytochemical analysis of the plant *Lawsonia inermis* (Henna) studied showed the presence of cardio glycosides, terpenoids and phenols. The oils and fats, tannins, quinones and saponins were absent in the plant *Lawsonia inermis*.

Earlier literature indicated that medicinal plants are the back bone of the traditional medicine and the antimicrobial activity of the plant extract is due to different chemical agent in the extract which were classified as active antimicrobial compounds **(Arulmozhi S *et al*., 2007).** These compounds attracts beneficial and repel harmful organisms so as photo protectants and respond to environmental changes **(Joy PP *et al*., 2001)**. Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores **(Dhar *et al*., 1979).**

ABBREVIATIONS:

N.A-Nutrient Agar, N.B- Nutrient Broth, O.D- Optical Density, Hrs-Hours, Conc- Concentration, mg –Mili gram, ml-Mili litre, mM- Mili molar, nm Nano metre, D/W-Distilled water, PDA- Potato Dextrose Agar, PDB- Potato Dextrose Broth, $^{?}$C- Degree Celsius, ZOI- Zone of inhibition, MIC- Minimum inhibitory concentration, MBC Minimum bacteriocidal concentration, MFC-Minimum fungicidal concentration.

CONCLUSION:

The present study and data revealed that the antimicrobial activity of *Lawsonia inermis* was found to be best against bacterial and fungus cultures and the phytochemial analysis showed the factors which are found in the form of secondary metabolites were responsible for antimicrobial activity. The future prospect for this study was to analyze the purified compound for drug validation in market through GC-MS and also with the help of PCR.

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