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Invitro Anti bacterial activity of Leaf Extracts OF *Lawsonia inermis*

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Abstract: The antibacterial activities of hexane, chloroform, methanol extracts of the leaves *Lawsonia inermis* (Henna) was evaluated on bacterial strains like *Escherichia coli, Klebsiella pneumonia, Lactobacillus acidophilus, Streptococcus aureus , Streptococcus mutans , Micrococcus , Streptococcus salivarius , Bacillus subtilis, Staphylococcus epidermidis, Streptococcus gordonii.*The in vitro antibacterial activity of leaf extracts was performed by agar cup plate / agar well diffusion method using streptomycin in dimethyl sulphoxide as a standard drug for the comparision of antibacterial activity. The results revealed that methanol extract shows lowest values of MIC when compared to other extracts. That means the highest anti bacterial activity exhibited by methanol extract. This study conclude that the methanol extract of leaf *Lawsonia inermis* showed more significant activity against all tested bacterial organisms than that of the hexane and chloroform extracts.
Key words: *Lawsonia inermis,* anti – bacterial activity, MIC, agar well diffusion method.

Introduction:

The synonyms are Lawsonia –alba, Henna

Henna or Hina (*Lawsonia inermis*, syn. *L. alba*) is a flowering plant, 2-6m in height. It is the sole species in the genus Lawsonia in the family Lythraceae [1]. Henna, *Lawsonia inermis*, produces a burgundy dye molecule, lawsone [2]. This molecule has an affinity for bonding with protein, and thus has been used to dye skin, hair, fingernails, leather, silk and wool. The dye molecule, lawsone, is primarily concentrated in the leaves. Products sold as "black henna" or "neutral henna" are not made from henna, but may be derived from indigo (in the plant Indigofera tinctoria) or Cassia obovata, and may contain unlisted dyes and chemicals.

It is well known that plants have been used in traditional herbal medicine for many years [3]. In some parts of the world, plants and herbs are still the prime medicines used in medical treatment [4,5]. L. inermis is widely grown in various tropical regions in Asia, America and Africa. In Arabic, the word "henna" refers to L. inermis [6, 7].

The main uses of henna are as a cooling agent, astringent, anti-fungal and anti-bacterial herb for the skin and hair. It has also been used as a dye and preservative for hair, skin and fingernails as well as leather and clothes [8, 9]. Its core chemical components are 2-hydroxynapthoquinone (lawsone), mannite, tannic acid, mucilage and gallic acid. Out of these

ingredients, the main one is 2-hydroxynapthoquinone (lawsone). About 0.5-1.5% of henna is made of lawsone. Its bioactive feature is thought to be due to its high protein binding capacity. It has tuberculostatic [10], hypoglycemic and hypolipidemic [11], wound healing [12] activities.

The different organisms used in this activity are *Escherichia coli, Klebsiella pneumonia, Lactobacillus acidophilus, Streptococcus aureus, Streptococcus mutans, Micrococcus , Streptococcus salivarius , Bacillus subtilis, Staphylococcus epidermidis, Streptococcus gordonii.* In this article, we wish to report the antibacterial activities of different leaf extracts of *Lawsonia inermis* against the above organisms.

Materials and Methods:

Collection of plant material:

The plant material was collected in Visakhapatnam from the Botanical garden of Andhra University. The plant material was thoroughly washed and dried in vacuum desiccators and was grinded to powder and 150gms of the dried plant material was used for the extraction.

Preparation of plant extract:

The collected plant material was taken and chopped into small pieces were shaded dried at room temperature (37^{o}C) for about a week. The coarsely powdered material weighed and extracted with chloroform, methanol, and hexane in sequential order of polarity using a soxhlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each gram of dry material 2ml of the solvent is used. The extracted solvents were filtered through whattman no-1 filter paper. The dried plant extract residues obtained were redissolved in 0.1% dimethyl sulfoxide (DMSO) to get different concentrations (50mg/ml, 100mg/ml, 300mg/ml, and 500mg/ml) of crude extracts and filtrations' through a 0.45 um membrane filter and stored in sterile brown bottles in a freezer at 20^{0}C until bioassayed.

Collection of Test Organisms:

Stock cultures of *Escherichia coli , Klebsiella pneumonia , Lactobacillus acidophilus , Streptococcus aureus Streptococcus mutans , Micrococcus , Streptococcus salivarius , Bacillus subtilis Staphylococcus epidermidis and Streptococcus gordonii.* These strains were obtained from MTCC and gene bank Chandigarh. Nutrient broth was used for growing and diluting the microorganisms' suspension.

Bacterial Susceptibility Testing:

The antibacterial activity of the chloroform, methanol and hexane of each sample was evaluated by using well diffusion method or couplet method of Murray et al., (1995) modified by olurinola, (1996), which is the most widely used type for identifying the antimicrobial activity, which exploits diffusion of antimicrobial compounds through agar media to demonstrate inhibition of bacteria.

Hexane, chloroform, methanol was dissolved in DMSO in each concentration of 1gm/ml. to liquefy the extract and used it for further analysis of antimicrobial activity.

Method: Agar cup plate method/ Agar well diffusion method

There are two methods agar disc diffusion and agar well diffusion. In these two methods the agar well diffusion assay [13, 14] was used to screen for antimicrobial activity of the chloroform, methanol and hexane extracts of different plant species. In agar well diffusion method peptone (0.5g), meat extract (1.0g), sodium chloride (0.5g), and agar (1.5g) were dissolved in small quantity of distilled water with the aid of heat on water bath and the volume was made up to 100ml. with purified water. The PH of the nutrient broth was adjusted to 7.2 with using 5M sodium hydroxide, and then sterilized in an autoclave, maintained at 121^{0} c (151 bs/sq.inch) for twenty minutes.

After sterilization the medium was inoculated with 3ul aliquots of culture containing approximately 10^{5} CFU/ml of each organism of 24 hrs slant culture in aseptic condition and transferred into sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes. After setting a number 3 cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each Petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups /wells were filled with 20Ul of the different extracts of 50mg / ml., 100mg /ml., 300mg /ml., and 500mg /ml., were allowed to diffuse plant extract into the medium for about 45 minutes.

Standard drugs streptomycin (20ug/ml), control (0.1%DMSO) was transferred to the cups of each agar plate by means of sterile pipettes under a laminar flow unit. The solvents used for reconstituting the extracts were similarly analyzed. The plates thus prepared were left for 2 hrs. In a refrigerator for diffusion and then kept in an incubator at 37^{0}C. After 24 hrs, the agar plates were examined for inhibition zones, and the zones were measured in millimeters and the results were given in tables.

Table 1: Antibacterial activity of Hexane extract of leaf Lawsonia *inermis*

Clinical pathogens	Zone of inhibition (mm)									
	500 mg/ml (8µl)	300 mg/ml (8µl)	100 mg/ml (8µl)	50 mg/ml (8µl)	25 mg/ml (8µl)	12.5 mg/ml (8µl)	6.25 mg/ml (8µl)	3.125 mg/ml (8µl)	Std 10mg/ml (5µl)	MIC mg/ml (8µl)
Escherichia coli	10	10	10	10	10	10	N.Z	N.Z	14	12.5mg/ml
Bacillus subtilis	10	10	10	10	10	10	10	N.Z	13	6.25mg/ml
Streptococcus salivarius	10	10	10	10	10	10	10	N.Z	17	6.25mg/ml
Micrococcus	10	10	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	16	300 mg/ml
Streptococcus mutans	10	10	10	10	N.Z	N.Z	N.Z	N.Z	16	50 mg/ml
Klebsiella pneumonia	11	10	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	21	300mg/ml
Staphylococcus epidermidis	13	12	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	20	300mg/ml
Lactobacillus acidophilus	10	10	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	20	300mg/ml
Streptococcus gordonii	10	10	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	17	300mg/ml
Streptococcus aureus	10mm	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	13	500mg/ml

Vol per well-20µl, Borer size used: 6mm, concentration of extracts- 3.125mg/ml,6.25mg/ml,12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml, 300mg/ml, and 500mg/ml: streptomycin-5µg/ml, where NZ is no zone

Table 2: Antibacterial activity of chloroform extract of leaf *Lawsonia inermis*

Clinical pathogens	Zone of inhibition (mm)									
	500 mg/ml (8µl)	300 mg/ml (8µl)	100 mg/ml (8µl)	50 mg/ml (8µl)	25 mg/ml (8µl)	12.5 mg/ml (8µl)	6.25 mg/ml (8µl)	3.125 mg/ml (8µl)	Std 10mg/ml (5µl)	MIC mg/ml (8µl)
Escherichia coli	12	11	11	10	N.Z	N.Z	N.Z	N.Z	14	50 mg/ml
Bacillus subtilis	12	12	11	10	10	10	10	N.Z	13	6.25mg/ml
Streptococcus salivarius	10	10	10	10	10	10	10	10	16	3.125mg/ml
Micrococcus	11	13	11	10	10	10	10	N.Z	17	6.25mg/ml
Streptococcus mutans	15	15	12	10	10	N.Z	N.Z	N.Z	16	25mg/ml
Klebsiella pneumonia	14	18	11	11	10	10	N.Z	N.Z	22	12.5mg/ml
Staphylococcus epidermidis	13	12	10	11	10	10	10	10	19	3.125mg/ml
Lactobacillus acidophilus	12	12	10	10	10	10	10	N.Z	15	6.25mg/ml
Streptococcus gordonii	14	12	11	10	10	N.Z	N.Z	N.Z	18	25mg/ml
Streptococcus aureus	10	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	N.Z	11	500mg/ml

Vol per well-20µl, Borer size used: 6mm, concentration of extracts- 3.125mg/ml,6.25mg/ml,12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml, 300mg/ml, 500mg/ml: streptomycin-5µg/ml, where NZ is no zone

Table 3: Antibacterial activity of methanol extract of leaf *Lawsonia inermis*

Clinical pathogens	Zone of inhibition (mm)									
	500 mg/ml (8µl)	300 mg/ml (8µl)	100 mg/ml (8µl)	50 mg/ml (8µl)	25 mg/ml (8µl)	12.5 mg/ml (8µl)	6.25 mg/ml (8µl)	3.125 mg/ml (8µl)	Std 10mg/ml (5µl)	MIC mg/ml (8µl)
Escherichia coli	12	14	10	10	10	10	N.Z	N.Z	14	12.5 mg/ml
Bacillus subtilis	13	14	11	10	10	10	N.Z	N.Z	13	12.5 mg/ml
Streptococcus salivarius	15	18	10	10	10	10	N.Z	N.Z	16	12.5 mg/ml
Micrococcus	17	13	12	11	10	10	N.Z	N.Z	15	12.5 mg/ml
Streptococcus mutans	18	17	12	11	10	10	N.Z	N.Z	18	12.5 mg/ml
Klebsiella pneumonia	13	12	12	10	10	10	N.Z	N.Z	N.Z	12.5 mg/ml
Staphylococcus epidermidis	16	14	14	12	10	10	10	10	18	3.125mg/ml
Lactobacillus acidophilus	13	13	10	10	10	10	N.Z	N.Z	22	12.5 mg/ml
Streptococcus gordonii	14	14	13	12	10	10	N.Z	N.Z	16	12.5 mg/ml
Streptococcus aureus	14	13	11	10	N.Z	N.Z	N.Z	N.Z	10	50 mg/ml

Vol per well-20µl, Borer size used: 6mm, concentration of extracts- 3.125mg/ml,6.25mg/ml,12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml, 300mg/ml, 500mg/ml: streptomycin-5µg/ml, where NZ is no zone

Results and Discussion:

Results of the antibacterial screening of different solvent extracts of *Lawsonia inermis* leaf revealed significant antibacterial activity against all tested bacterial strains. The order of potency of the three extracts are in the following order, methanol > chloroform > hexane. The methanol extract exhibited a greater anti-bacterial activity against *Bacillus subtilis, Streptococcus salivarius, Micrococcus, Klebsiella pneumonia, Streptococcus aureus* when compared with standard antibiotic. Henna contains Lawsone in about 0.5 to 1.5% of its ingredients. Lawsone (2-hydroxynapthoquinone) is the principal constituent responsible for the dyeing properties of the plant. However, henna also contains mannite, tannic acid, mucilage and gallic acid. These substances are present in henna in the form of a mixture. Anti-bacterial activity 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. The methanolic extract showed the lowest MICs compared to other types of extracts and this may be due to the large quantity of active substances that were precipitated during the extraction process. That means when compared to methanol extract the hexane and chloroform extracts precipitate less quantity of active constituents during extraction process. The minimum inhibitory concentrations of these extracts are also very high when compared to methanol extract. Results revealed that the hexane extract act against *Streptococcus aureus* only at high concentrations. It is very effective against *Bacillus subtilis, Streptococcus salivarius* when compared to other organisms. The chloroform extract is somewhat more effective when compared to hexane extract and it shows maximum effect against *Streptococcus salivarius, Staphylococcus epidermidis*. The methanol extract is very effective against all organisms when compared to other extracts. We concluded that henna has an in-vitro anti-bacterial activity against the tested bacterial strains. These findings have also been mentioned in literatures.

Conclusion:

The results obtained in this study conclude that the methanol extract of leaf *Lawsonia inermis* showed more significant activity against all tested bacterial organisms than that of the hexane and chloroform extracts. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

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