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Comparative antibacterial studies of *in vivo* and *in vitro* leaves of *Lawsonia inermis* L.- A multipurpose medicinal plant

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ABSTRACT

A comparative antibacterial study of *in vivo* and *in vitro* leaf extracts was performed against four different bacterial strains viz., *Streptococcus pyogenes, Streptococcus mutans*, *Shigella flexnerii* and *Salmonella enteric-typhii*. Both types of leaf materials (*in vivo* and *in vitro*) of *L. inermis* screened for four different solvent systems, showed anti-bacterial activity (MIC values) against all human pathogenic bacteria tested. Of the four bacterial strains evaluated, the best MIC value (200 µg/ml) was observed in *in vitro* leaf extract of all the solvent system against *Streptococcus pyogenes*. Acetone and n-hexane *in vitro* leaf extract was found to be more effective (MIC = 200 µg/ml) than *in vivo* leaf extract (MIC = 400 µg/ml) for *S. pyogenes* and *S. mutans,* respectively. The results of this study tend to give credence to the use of henna leaf of *in vitro* regenerated plants as antibacterial agent in pharmaceutical industries without disturbing the garden plants.

1. Introduction

Lawsonia inermis L. (Family - Lythraceae) is an important multipurpose medicinal plant, , which is commonly known as Henna or Mehendi or Manjuati. The plant is distributed over northern Africa and south–west Asia (Chand and Jangid, 2007). In India it is cultivated as cash crop particularly in Sojat area of Pali district, Rajasthan (Ram and Shekhawat, 2011). The plant have immunomodulatory, antiviral, antibacterial, antifungal, antifertility, hepatoprotective, anticancer, analgesic and anti-inflammatory properties (Chowdhury *et al.,* 2010). Leaves are also useful to bring down the severity of many medical problems like diarrhea, dysentery, gastric pain, jaundice, diseases of the spleen, lumbago, bronchitis and syphilitic, eye infections, etc. Some of the tribal use the plant leaves for the treatment of body ache, skin infections, inflammations, urinary tract infection, allergy etc. (Bellakhdar, 1997; Ahmed *et al.,* 2000; Bhuvaneswari *et al.,* 2002; Lahsissene and Kahouadji, 2010).

The leaf of *L. inermis* is an important plant part and many workers have studied the effect of different solvent based leaf extracts on different bacterial strains like *Streptococcus aureus* and *Streptococcus epidermidis* (Al-Rubiay *et al.,* 2008), *Streptococcus aureus, Bacillus subtilis* and *Estricheria coli* (Nagarajan *et al.,* 2013), *Micrococcous luteus* and *Pseudomonas aeruginosa* (Dhanalakhmi *et al.,* 2013). Based on these observations, the present study was undertaken to determine the possible antibacterial activities of *L. inermis* leaf extracts on human pathogenic bacterial strains such as *Streptococcus pyogenes, Streptococcus mutans, Salmonella enteric-typhii* and *Shigella flexneri* among which *S. pyogenes* is the most common bacteria causing sore throat (Rout *et al.,* 2001; Duckworth, 2006).

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Further, it is assumed that the demand of drug industries can be meet by the use of *in vitro* regenerated plant parts. Keeping this in view in this paper, we are describing the screening of different extracts of *in vivo* and *in vitro* leaves for antibacterial activities with a conclusion that *in vitro* plant parts can be efficient and alternative source for the drug industries.

2. Materials and methods

Two different types of leaf materials i.e. *in vivo* (leaves from garden grown plant in the Dept. of Botany, Ravenshaw University, Cuttack) and *in vitro* (leaves from plant tissue culture derived plants maintained in the garden of the Dept. of Botany, Ravenshaw University, Cuttack) of *L. inermis* were used for the antibacterial studies. Four different solvents as per their polarity index [n-hexane, acetone, methanol; (Merck, India) and aqueous] were used for the extraction and screening of antibacterial activity. For the study, bacterial strains including two Gram-positive bacteria namely *Streptococcus pyogenes* (MTCC-1926) and *S. mutans* (MTCC-497) as well as two Gram-negative bacteria namely *Salmonella enteric typhii* (MTCC-1252), *Shigella flexnerii* (MTCC-1457) were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) using serial dilution method (CLSI, 2002). Selected colonies of aforesaid bacteria were picked off from a fresh isolation plate and inoculated in corresponding tubes containing 5 ml of trypticase soya broth (Hi-media, Mumbai). The broth was incubated for 6±1 hours at 35±2 ^{0}C until there was visible growth. Mc Farland 0.5 standard was used to adjust the turbidity to get 10^5 colony flow per unit (cfu)/ml. Each crude leaf extracts of 8000 µg was dissolved in 10 ml of trypticase soya broth for bacterial growth to get 800 µg/ml drug concentrations.

MIC was calculated by two fold serial broth dilution for four different leaf extracts/solvents with a standard Kanamycin (Hi-media, Mumbai) for all four bacterial strains. The method includes 21 tubes of 5 ml capacity were arranged in 3 rows/replications with each row containing 7 tubes. Trypticase soya broth of 1.9 ml to first tube and 1ml to other 6 tubes was added in each row or to replication. Leaf extract suspension of 100 µl was added to the first tube in each row and after mixing the content and then serial dilution was made to achieve the concentrations of 800, 400, 200, 100, 50, 25 and 12.5 µg/ml. The tubes were inoculated with 1 ml (10^5cfu/ml) of bacterial suspension and then incubated for 12-18 hours at 35±2 °C.

After the incubation, the tubes of lowest concentration showing no visible growth after 8 hours till 12 hours were considered to be inhibition of bacteria which represent MIC values of a respective concentration. Triplicates were maintained and the experiment was repeated thrice, for each replicates the average readings were taken for all the experiments designed.

3. Results and discussion

Both *in vivo* and *in vitro* leaf materials of *L. inermis* screened with four different solvent systems (n-hexane, acetone, methanol and aqueous) showed antibacterial activity (MIC values) against all human pathogenic bacterial strains (Fig. 1 and 2). Similar results have been obtained earlier in medicinal plants like *Tinospora cordifolia* (Kumari, 2012) and *Tylophora indica* (Jahan *et al.,* 2013).

Among the four solvents screened, n-hexane leaf extract of both *in vivo* and *in vitro* showed excellent antibacterial activity against all used pathogenic strains followed by aqueous extract. Highest antibacterial activity (MIC = 200µg/ml) was observed in all solvent extracts of both *in vitro* and *in vivo* leaf against *S. pyogenes* except acetone *in vivo* leaf extract (MIC=400 µg/ml). The lowest antibacterial activity (MIC = 800 µg/ml) was observed in acetone and methanol extract of both the leaf against *S. flexnerii*. Kanamycin was used as standard for bacterial strains with highest MIC at 25µg/ml and a lowest MIC 12.5µg/ml (Fig. 1 and 2).

Available literature indicates that the human pathogenic bacteria *S. pyogenes* are becoming resistance to antibiotics (Gracia *et al.,* 2009; Malli *et al.,* 2010) and causing the most common sore throat (Duckworth, 2006). Our results revealed that the henna leaf extract of both the sources has potential for controlling the bacterial strain *S. pyogenes*. This also validates a medicinal claim that gurgling of henna leaf extract can cure sore throat as reported by Rout *et al.* (2001). Use of *L. inermis* leaves against *S. pyogenes* for skin diseases has also been reported (Chaudhary *et al.,* 2010).

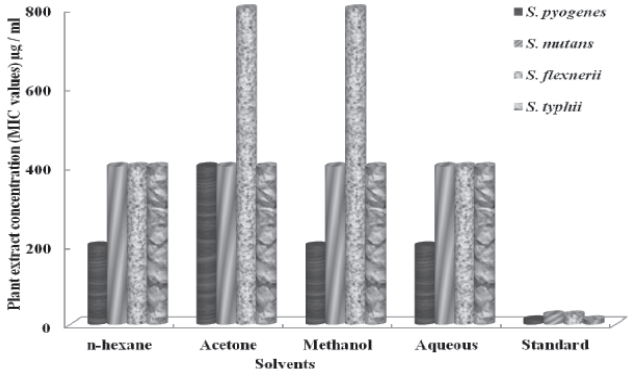

Fig. 1 Comparative antibacterial activity (MIC values) of *in vivo* leaf extracts of *L. inermis*

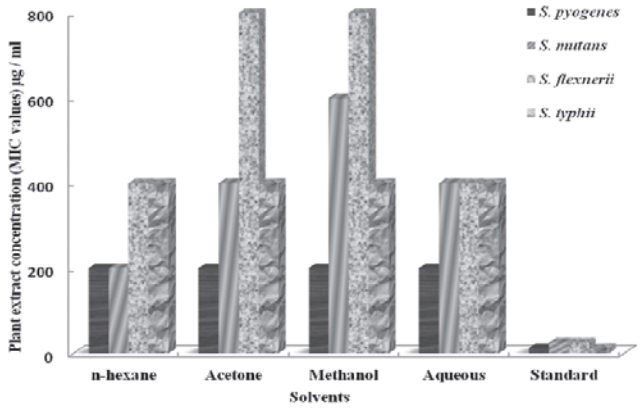

Fig. 2 Comparative antibacterial activity (MIC values) of *in vitro* leaf extracts of *L. inermis*

Papageorgiou *et al.* (1999) observed that *L. inermis* leaf extracts exhibit antibacterial activity only against gram positive bacteria while ineffective for gram negative bacteria but we observed antibacterial activity against both gram positive and gram negative bacteria. Similar results were obtained by Hussain *et al.* (2011) and Gull *et al.* (2013).

Our results showed the potential antibacterial activity of *in vitro* leaf extracts which is comparable to *in vivo* leaf extracts. Antibacterial test in this study against known human pathogens and its action required the evaluation of the pharmacological values of above extracts to fight against antimicrobial resistance and infections as well as formulation of new drugs from *in vitro* plant parts.

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