Antibacterial Activity of *Lawsonia inermis* L.

M. Nagarajan, S. Rajasekaran, K. Sankar Ganesh*

*Department of Botany, A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai-609305, India*

*Author to whom correspondence should be addressed; E-Mail: indsankar@rediffmail.com.*

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**Abstract:** The screening of antibacterial activity of traditionally used medicinal plant *Lawsonia inermis* leaves in different extracts such as ethanol, chloroform, methanol and hexane was studied against two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and one Gram negative bacteria (*Echerichia coli*) by agar well-diffusion method. Four dilutions, viz., 1000, 2000, 3000 and 4000 µg/µL were made for the extract and the zones of inhibitions were increased with the concentration of the extracts. At 4000 µg/µL, the plant extracts showed nearly equal zone to that of standard antibiotic used. Among all the extracts used, ethanol extract of *Lawsonia inermis* showed best activity against test pathogens, when compared to other extracts. Others showed moderate activity.

**Keywords:** *Lawsonia inermis*; leaf; bacteria; inhibition zone; antibacterial activity.

**1. Introduction**

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Nowadays, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Ellof, 1998). The use of crude extracts of plants parts and phytochemicals of known antimicrobial properties can be of great significance in
the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. The screenings of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes (Selvamohan et al., 2012). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants (Wu et al., 1999). Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants, and out of the 400,000 plant species on earth, only a smaller number has been systematically investigated for their antimicrobial activities (Anjana et al., 2009).

Medicinal plants have been known for millennia, and all cultures from ancient times to present day have used plants as a source of medicine. The recent studies also import the identification and isolation of new therapeutic compounds of medicinal importance from the higher plants for specific diseases. In order to find out new sources of drugs, a number of plants have been screened for wide range of biological activities in India. Medicinal herbs possess curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants (Patil et al., 2009). There is continuous and urgent need for discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because of alarming increase in the incidence of new and re-emerging infectious diseases. Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs (Kamaraj et al., 2012). In this context, the status of antimicrobial activity of widely used traditional medicinal plants in India, *Lawsonia interims* Linn. (Lythraceae) which have been used for several purposes including treatment of various diseases has studied.

It is a biennial dicotyledonous herbaceous shrub (Plate 1). A native of North Africa and South-West Asia, the plant is now widely cultivated throughout the tropics as an ornamental and dye plant. Young branches are green in colour and quadrangular which turn red with age. Bark is greyish brown, unarmed when young but branches of older trees are spine tipped. Inflorescence is a large pyramid.
shaped cyme. Flowers are small, numerous, fragrant, white or rose coloured with four crumbled petals. Fruit is a small brown coloured round capsule. Fruit opens irregularly and splits into four sections at maturity and is many seeded. Seeds are about 3 mm across, numerous, smooth, pyramidal, hard and thick seed coat with brownish coloration.

Plate 1. Morphology of *Lawsonia inermis* L.

Henna has been used cosmetically and medicinally for over 9,000 years. Traditionally in India, mehandi is applied to hands and feet. Henna symbolizes fertility. Its use became popular in India because of its cooling effect in the hot Indian summers. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrhea, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent (Choudhary et al., 2010).

2. Materials and Methods

The test microorganisms namely, *Bacillus subtilis*, *Staphylococcus aurous*, and *Escherichia coli* were isolated from the wound exudates, which were collected from the hospitals were identified. The isolated and identified bacterial pathogens were further sub-cultured on their corresponding selective media and stored on nutrient agar slants. These pathogens were used as test organisms for *in vitro* screening of antibacterial activity of the medicinal plants.

The leaves of the plants were collected and dried separately and powered in a Wiley mill. Leaf
extraction was made by cold maceration method. Ethanol, chloroform, hexane and methane were used as extraction solvents. Fifty gram of dried leaf powder was added with 400 mL of each solvent and filtered. The filtrate was evaporated using hot plate. The dried crude extracts were dissolved in 5% dimethyl soleplate. From the stock solution, four different concentrations of each crude extract were prepared with distilled water (1000, 2000, 3000 and 4000 µg/µL respectively). The extracts were tested for its antibacterial activity by well diffusion method. The enriched cultures were swabbed on to the sterilized Mullen-Hinton Agar plates by using sterile swabs. With the help of a well puncher, five wells were made in each plate by 27 mm apart. The diameter of each well was 9 mm. Wells were filled with 100 µL of prepared dilution (1000, 2000, 3000 and 4000 µg/µL) of plant extract. One well was filled with standard antibiotic solution (tetracycline 100 µg/µL) as positive control. The antibacterial activity of each crude extract was measured in terms of inhibitory zones formed around the well in millimeters.

3. Results and Discussion

The results obtained are given Table 1. Traditional medicine has been practiced for many centuries by sustainable proportion of Indian population. The interest in the study of medicinal plant as a source of pharmacological active compounds has increased worldwide (Sagayaraj et al., 2001). It is recognized some developing countries, that plants are the medicinal source of treat infectious diseases. The world is emerging in the grip of dreadful fear of development of resistance to antibiotics by the microorganisms. For the treatment of infectious, finding out the substitute from the nature to the antibiotics is becoming the prime need of society in the present and in future. Higher plants often immense possibilities of discovering potent drugs including those active against infectious diseases. It is expected that plants should possess chemicals that are active against a variety of essential enzymes possessed by the bacteria, fungi and protozoa. Medicinal plants are rich sources of therapeutic agents without serious side effects, for prevention and cure of various diseases of human beings.

Table 1. Antibacterial activity of \textit{Lawsonia inermis} in various extracts

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Zone of Inhibition (mm) in various extracts</th>
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<tbody>
<tr>
<td></td>
<td>Ethanol (× 10^3 µg/µL)</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>19  22  23  23</td>
</tr>
<tr>
<td>S. aureus</td>
<td>16  16  18  20</td>
</tr>
<tr>
<td>E. coli</td>
<td>12  13  14  16</td>
</tr>
</tbody>
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Note: \textit{B. subtilis}-\textit{Bacillus subtilis}, \textit{S. aureus}-\textit{Staphylococcus aureus}, and \textit{E. coli}-\textit{Escherichia coli}.
Antimicrobial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, with the ‘antibiotic era’ barely five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens (Peterson and Dalhoff, 2004). During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Lanfranco, 1999). The herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality (WHO, 2001).

In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new antimicrobial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of the commonly used antibiotics (Abebe et al., 2003). Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified. It is believed that plant chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates are generally superior in their antimicrobial activities (Cowan, 1999).

The plant showed antibiotic activity against the test organisms used. The zone of inhibition was increased with increase in the concentration of extracts. At 4000 µg/µL, among all the plant extracts of *lawsonia inermis*, ethanol extract showed the maximum antibacterial activity against all the test organisms. This indicates that most of the active compounds in the plant might be soluble in particular solvent then other solvents (Choudhary et al., 2010). *Bacillus subtilis* and *staphylococcus auruas* were highly susceptible in all the four extracts of the plant. Thus, the study reveals that the plant used have antimicrobial substances, but its susceptibility varies with various solvent extracts, and it is clear that the leaves of *Lawsonia inermis* as an extract or suspension or any modified formulation may be useful as an antimicrobial agent against these pathogens causing infections. In this juncture traditional medicinal plants must perforce be granted the benefits of modern scientific technology to serve further global needs. This dried form of herbs may have the possibility of their use in medicine because of their fine antimicrobial activity.

4. Conclusions

At 4000 µg/µL, the plant extracts showed nearly equal zone to that of the standard antibiotic. Among all the extracts, ethanol extract of *Lawsonia inermis* showed the best activity against test pathogens, when compared to other extracts. Others showed moderate activities.
References


