

Research Article

## Studies on antimicrobial activity of *Lawsonia inermis* L. against different strains of bacteria and fungi

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### Abstract

Natural plant products are a significant source of synthetic and traditional medicines. The majority of the world population, especially in developing countries, is dependent on herbal formulations for their primary health care needs. *Lawsonia inermis* is a popular medicinal plant and possess many pharmacological properties. The present study was carried out to estimate the antimicrobial activity of *L. inermis* leaves of the mother plant, regenerated plants and callus extract to justify the pharmaceutical aspect of the plant to provide herbal plant products as phytochemistry in Ayurveda as well as ethnobotanical aspect of the plant. The antimicrobial activity was determined with the help of the agar well diffusion method by using some Gram-positive; *Bacillus subtilis* MTCC441, *Bacillus cereus* MTCC430, *Staphylococcus aureus* MTCC96, Gram-negative; *Escherichia coli* MTCC1885, *Pseudomonas aeruginosa* MTCC424 and fungal strains *Candida albicans* MTCC227. Leaves and callus extract were tested against these microorganisms in different types of solvent as Methanolic, Ethanolic, Aqueous, Acetone, Hexane, Chloroform and Diethyl ether were investigated by agar well diffusion method. Different extraction procedures were done by using the soxhlet apparatus. Dilutions were made for the extract and it was noticed that the zones of inhibitions were increased with the concentration of the extracts. Methanolic leaves extract was found best extract for antimicrobial activity of *L. inermis* in comparison to other extracts. The maximum zone of inhibition was  $13.79 \pm 1.7$  mm in methanolic extracts of leaves against *B. subtilis* and the minimum zone of inhibition was noticed  $09.40 \pm 1.7$  mm against *B. cereus*. The value of the zone of inhibition was more in the case of leaves extracts as compared to callus extracts. This study showed that methanolic leaves extracts of *L. inermis* inhibit the growth of microorganisms dose-dependently. The leaves of *Lawsonia inermis* support the traditional use of the plant in therapy of bacterial infection.

**Keywords:** Antimicrobial, Agar well diffusion method, Bacteria, Fungi, *Lawsonia inermis*, Phytochemicals

### INTRODUCTION

Bacteria and fungi have the phylogenetic capacity to cause the disease and acquire resistance to drug, which is obtained from plants are utilized as curative agents (Fair and Tor, 2014). Though pharmacological industries are producing a number of new broad-spectrum antibiotics in the last three decades, resistance to these drugs by microorganisms has increased to a considerable extent. Various bacteria and fungi are extremely pathogenic, causing infectious ailments (Prestinaci *et al.*, 2015). The discovery of antibiotics to fight these pathogens was marked as an aim in the 20<sup>th</sup> century. *Lawsonia inermis* belongs to the family Lythraceae. It is distributed in Punjab, Gujarat, Haryana

and Rajasthan (Chand and Jangid 2007). In world, *Lawsonia inermis* (heena) distribution is in the Middle East, Northern Africa, and southwest Asia along the coast of the sea and Mediterranean sea (Hutchison and Dalziel, 1954). It is widely distributed as an ornamental hedge shrub plant. This plant has been used for centuries, in many traditional aspects, likewise occasionally mainly as a dye for hair and nail and hands in an artistic way. This plant has antimicrobial, antimycotic, antiparasitic, and virucidal activity due to its major bioactive constituents like mannite, tannic acid, mucilage and gallic acid, and 2-hydroxynaphthoquinone (lawsone) (Muhammad and Muhammad 2005, Charoensup *et al.*, 2017). Traditional medicinal uses of heena are in healing dysentery, spleen diseases, bronchi-

tis, and syphilitic eye infection and for the skin and hair health. Many plants have been used because of their antimicrobial activity, which are due to the secondary metabolites synthesized by the plants. These products are known for their active substances like phenolic compounds, which are part of the essential oils and 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, Usman and Rabi, 2018). Due to the high medicinal value of the plant, an attempt was made to find out the antimicrobial potential of leaves and callus extracts. These extracts were tested against bacteria and fungi in different types of solvent as methanolic, ethanolic, aqueous, acetone, hexane, chloroform and diethyl ether and were investigated by using the agar well diffusion method.

## MATERIALS AND METHODS

### Preparation of the extract

Distilled water was used to cleaning of leaves and these were scorched using hot air oven for about four to seven days at 50 °C temperature. The dried leaves and callus were powdered in a pestle mortar. Methanolic, petroleum ether, ethyl acetate and aqueous extract of leaves and callus were prepared. 50 gm of materials were extracted with 250 ml of corresponding solvents in a soxhlet apparatus at room temperature for about 24-72 hours. The extract was sieved through filter paper (Whatman no. 1). The residue was dried and used. Nutrient agar / Broth was used for bacterial growth and maintenance to test the antibacterial assay (Mueller and Hinton, 1941).

### Bacterial and fungal test strains used for antimicrobial assay

The different bacterial test strains used for agar well diffusion method plate antimicrobial assay are listed in Table 1. The reference fungal and bacterial strains used in this study were obtained from the Council of Scientific & Industrial Research – Institute of Microbial Technology, CSIR IMTECH Chandigarh. The bacterial and fungal cultures were maintained on Nutrient agar (NA) and PDA media by regular transfers. For fungal

cultures, the suspension was made by adding 0.4 ml sterile water and allowed to stay for 20 minutes before transferring on to solid medium. A few drops of the suspension were streaked on to PDA medium and incubated at 25 c for two days. The purity of cultures was confirmed by preparing a smear and looking under the microscope from time to time. The comparative antibacterial activities of callus extract and root extract in different types of solvents were effectively accessed against three gram-positive bacteria, two gram-negative bacteria and one fungal strains as test microorganisms using agar well diffusion method.

Methanolic, Ethanolic, Aqueous, Acetone, Hexane, Chloroform and Diethyl ether extracts were investigated by agar well diffusion method. Extraction was carried out by a simple blender to grind the dried leaves with various solvents and then a series of centrifuges to eliminate the solid waste. After that, through a series of filtrations, a sterile extract was prepared. Different extraction procedures were done by using Soxhlet apparatus. Powdered leaves or callus materials were placed in soxhlet thimble. Solvent was then heated under reflux. Condensation and extraction was carried out with fresh solvent. Solutes were finally transferred from the extraction chamber into the reservoir. Continuous repetition of the extraction was done.

### Antimicrobial activity evaluation

Agar well diffusion method established by Perez *et al.* (1990) was employed for the evaluation of potentiality against microbial growth. For fungi and bacteria, Mueller-Hinton agar and Nutrient agar plates were swabbed with about one day old broth culture respectively. The inoculates were permitted to dryness for a duration of about five to ten min. Decontaminated cork borer was used for making the well (approximately 10mm diameter) in the culturing plates. Around one hundred micro litre, aliquot of both the plant leaves and callus extracts were poured separately in these wells. These plates were then incubated at 28 degree Celsius temperature for about 48 hrs duration for the fungal strain of *Candida albicans* and at 37 degree Celsius for just about 18-24 hrs of duration for the *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (bacterial pathogens). 0.1ml of 20µg/ml Ciprofloxacin and Ketoconazole were used as controls for the bacterial and fungal plates respectively.

### Minimum inhibitory concentration (MIC)

As per definition, the minimum inhibitory concentration indicates the lowest substance concentration, which severely reduces microbial growth. The Serial Dilution Method was employed for evaluating the MIC (Natta *et al.*, 2008). The stock solution was made by the dissolu-

**Table 1.** Microorganisms used in antimicrobial assay

Test Organisms	Nature	Strain No.
<i>Bacillus cereus</i>	Gram +ve	MTCC 430
<i>Bacillus subtilis</i>	Gram +ve	MTCC 441
<i>Escherichia coli</i>	Gram -ve	MTCC 1885
<i>Pseudomonas aeruginosa</i>	Gram -ve	MTCC 424
<i>Staphylococcus aureus</i>	Gram +ve	MTCC 96
<i>Candida albicans</i>	Fungi	MTCC 227

tion of two hundred mg of leaves and callus material into the 1ml Dimethyl sulfoxide. A number of dilutions (100 mg/ml, 50 mg/ml 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12 mg/ml, 1.56 mg/ml and 0.78mg/ml) were prepared from the stock solution and were then poured in the cultured plates containing 2.0ml nutrient broth for bacteria and PDA for fungal strains. 0.1ml of bacterial and fungal inoculum was added to each test tube. These were incubated at thirty-seven degree celsius temperature for twenty-four hours in a restrained undisturbed state. The lowest concentration of extracts that produced no visible growth (turbidity) was recorded as MIC. Tubes with broth and extracts without inoculum served as positive control whereas tubes with broth and inoculum without extracts were used as the negative control.

## RESULTS

The antibacterial and antifungal activity of aqueous, ethanolic, methanolic, diethyl ether, n-hexane and chloroform extracts of leaves and callus of *L. inermis* are tabulated in Table 2 and 3. The results showed that all the extracts exhibited a prohibitory role in the tested microbes' growth.

The aqueous leaves and callus extract exhibited a maximum interdictory role on the growth of *B. subtilis*, whereas minimum inhibitory action on the growth of *Staphylococcus aureus*. The maximum zone of inhibition was  $09.41\pm 1.1$ mm in aqueous extracts of leaves against *B. subtilis* and the minimum zone of inhibition was noticed  $07.20\pm 0.8$ mm against *S. aureus*. Same trend was noted on aqueous extracts of callus. The value of the zone of inhibition was more in the case of leaves extracts in comparison with extracts of callus. Leaves and callus ethanol extract exhibited a maximum interdictory role on the growth of *B. subtilis* (Fig. 2), whereas minimum inhibitory action on the growth of *Pseudomonas aeruginosa*. The maximum zone of inhibition was  $10.62\pm 1.3$  mm in ethanol extracts of leaves against *B. subtilis* and the minimum zone of inhibition was noticed  $08.24\pm 1.4$ mm against *P. aeruginosa*. Similar observations were noted on ethanolic extracts of callus. The value of the zone of inhibition was more in the case of leaves extracts compared to ethanol extracts of callus.

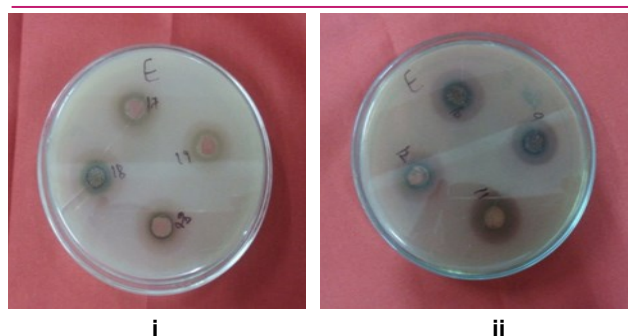
The methanolic callus and leaves extract exhibited a maximum prohibitory role on the growth of *B. subtilis*, whereas a minimum prohibitory role on the growth of *B. cereus*. The maximum zone of inhibition was

**Table 2.** Antimicrobial activity of leaves extract of *L. inermis*

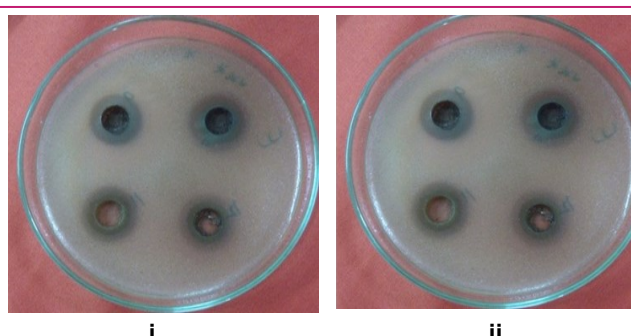
Type of Extracts	Zone of Inhibition (mm) Mean					
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Candida albicans</i>
Aqueous	07.20±0.8	09.41±1.1	08.11±1.6	07.30±1.6	08.22±1.6	07.43±1.2
Ethanolic	09.11±1.1	10.62±1.3	09.65±1.4	08.24±1.4	08.85±1.1	07.61±1.5
Methanolic	11.39±0.8	13.79±1.7	10.52±2.2	09.62±1.5	09.40±1.7	08.26±1.3
Diethyl ether	09.50±2.2	11.18±2.1	09.28±1.6	08.68±1.4	08.72±2.2	07.67±1.8
n-Hexane	09.15±0.8	09.61±1.6	08.21±1.4	08.15±1.2	08.43±1.2	07.14±1.3
Chloroform	08.02±1.2	09.90±1.7	08.17±1.2	08.20±2.2	08.11±1.3	07.24±1.5
Control	25.0±2.0	22.0±1.5	22.0±1.0	21.0±1.5	20.0±2.0	17.0±1.0

**Table 3.** Antimicrobial activity of callus extract derived from internodal explants of *L. inermis*

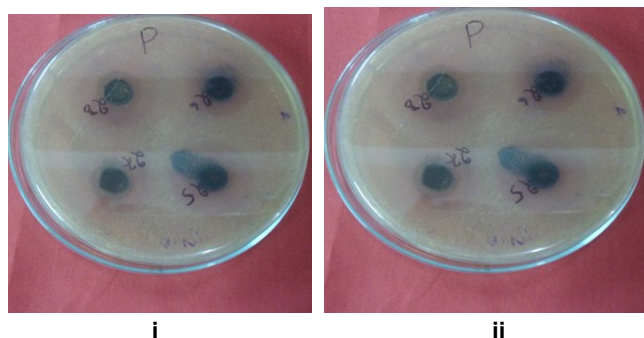
Type of Extracts	Zone of Inhibition (mm) Mean					
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Candida albicans</i>
Aqueous	05.25±1.0	07.45±1.2	06.15±1.7	05.31±1.8	06.25±1.5	05.42±1.3
Ethanolic	07.10±1.3	08.61±1.3	07.63±1.5	06.25±1.4	06.87±1.1	05.64±1.6
Methanolic	09.40±0.7	11.80±1.8	08.50±2.1	07.61±1.6	07.41±1.8	06.25±1.4
Diethyl ether	07.51±2.3	09.20±2.2	07.26±1.7	06.70±1.5	06.70±2.1	05.65±1.9
n-Hexane	7.15±0.9	07.60±1.5	06.21±1.5	06.18±1.1	06.42±1.3	05.12±1.4
Chloroform	06.00±1.1	07.92±1.8	06.16±1.1	06.19±2.1	06.10±1.4	05.23±1.6
Control	25.0±2.0	22.0±1.5	22.0±1.0	21.0±1.5	20.0±2.0	17.0±1.0



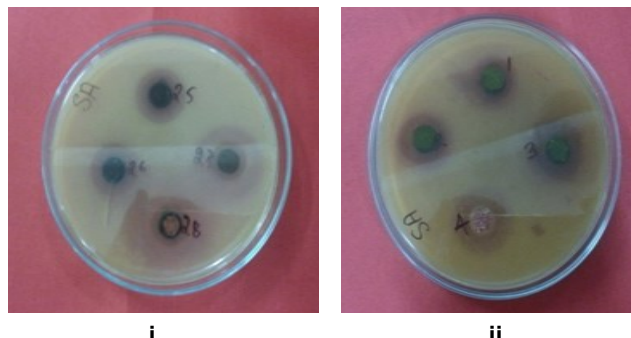
**Fig. 1.** Antibacterial activity of leaves (i) and callus extracts (ii) against *E. coli*



**Fig. 2.** Antibacterial activity of leaves (i) and callus extracts (ii) against *B. subtilis*



**Fig. 3.** Antibacterial activity of leaves (i) and callus extracts (ii) against *C. albicans*



**Fig. 4.** Antibacterial activity of leaves (i) and callus extracts (ii) against *S. aureus*

13.79±1.7mm in methanolic extracts of leaves against *B. subtilis* and the minimum zone of inhibition was noticed 09.40±1.7mm against *B. cereus*. Similar observations were noted on methanolic extracts of callus. The value of the zone of inhibition was more in the case of leaves extracts as compared to callus extracts. Similar observations were recorded for diethyl ether and n-hexane extracts of leaves and callus against the tested bacterial strains.

Chloroform extracts of leaves and callus recorded higher inhibitory activity in *B. subtilis* and lower in *S. aureus* (Fig. 2 and 4). The maximum zone of inhibition was 09.90±1.7mm in chloroform extracts of leaves against *B. subtilis* and the minimum zone of inhibition was noticed 08.02±1.2mm against *S. aureus*. Similar observations were noticed on chloroform extracts of callus. The value of the zone of inhibition was more in the case of leaves extracts in comparison with callus extracts.

Extracts of leaves and callus have shown the inhibitory growth action against the tested fungal strain *Candida albicans*. The methanolic extracts were more effective than others with an inhibitory zone of 08.26±1.3 mm. Leaves extracts proved to be better than the callus extracts. The value of the zone of inhibition was more in the case of leaves extracts than callus extracts. Antifungal assays showed that a maximum zone of inhibition (08.26±1.3mm) was observed in methanolic extracts against *C. albicans* ( Fig. 3 ).

The minimum inhibitory concentration of plant and callus extracts against different types of microorganisms

are tabulated in Table 4. The results obtained from this study showed that the leaf and callus extract of the plant inhibited the growth of the test isolates at varying concentrations. It revealed that the requirement of the extract was least in the case of *B. cereus* (1.6 and 2.9 mg/ml) and it was high in *P. aeruginosa* (3.6 and 7.1 mg/ml) in both the callus and leaves extracts.

## DISCUSSION

Due to the increase in broad utilization of anti-infection agents, the resistance capacity of various microorganisms is enhancing day by day. It results in a magnification of the morbidity and mortality rate and increases the cost of the medical services (Mahesh and Satish, 2008). The development of multidrug resistance strain has influenced the investigation of safe, new and herbal origin medicines. Among natural resources, phytoconstituents are a great source of antimicrobial compounds that suppress the growth of microorganisms, present either in an active form or activated by the presence of microbes or tissue injury. According to Ali *et al.*, 2016, it was found that the leaves of *L. inermis* are an excellent source of secondary metabolites and act as a robust antimicrobial agent against various microbial strains such as *Escherichia faecalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Some workers have reported that antimicrobial constituents of the plant extracts (terpenoid, alkaloid and phenolic compounds) react with the microbial membranous enzymes and pro-

**Table 3.** Antimicrobial activity of callus extract derived from internodal explants of *L. inermis*

Type of Extracts	Zone of Inhibition (mm) Mean					
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Candida albicans</i>
Aqueous	05.25±1.0	07.45±1.2	06.15±1.7	05.31±1.8	06.25±1.5	05.42±1.3
Ethanollic	07.10±1.3	08.61±1.3	07.63±1.5	06.25±1.4	06.87±1.1	05.64±1.6
Methanolic	09.40±0.7	11.80±1.8	08.50±2.1	07.61±1.6	07.41±1.8	06.25±1.4
Diethyl ether	07.51±2.3	09.20±2.2	07.26±1.7	06.70±1.5	06.70±2.1	05.65±1.9
n-Hexane	7.15±0.9	07.60±1.5	06.21±1.5	06.18±1.1	06.42±1.3	05.12±1.4
Choloroform	06.00±1.1	07.92±1.8	06.16±1.1	06.19±2.1	06.10±1.4	05.23±1.6
Control	25.0±2.0	22.0±1.5	22.0±1.0	21.0±1.5	20.0±2.0	17.0±1.0

**Table 4.** Minimum inhibitory concentration of plant extracts against different types of microorganism (mg/ml)

Sr No.	Microorganism	Leaf (mg/ml)	Callus (mg/ml)
1.	<i>E. coli</i>	1.9	3.4
2.	<i>S. aureus</i>	2.5	4.5
3.	<i>P. aeruginosa</i>	3.6	7.1
4.	<i>B. subtilis</i>	2.2	3.5
5.	<i>C. albicans</i>	3.1	5.7
6.	<i>B.cereus</i>	1.6	2.9

teins. It resulted in the disruption of the membrane to disperse a flux of protons towards the outside. This initiates cell death or may inhibit the enzymes essential for the biosynthesis of amino acids (Gill and Holley, 2006). Other scientists ascertain the inhibitory actions of these plant extracts to hydrophobic properties of these plants extracts. This property allows them to react with cell and mitochondrial membrane proteins. Therefore, disturb the structures of membranes and change their permeability (Tiwari *et al.*, 2000, Friedman *et al.*, 2004).

The most common method used for the antimicrobial assay is agar well diffusion, which has been used by many researchers on various parts of plant species and reported that these control the growth of *S. aureus* in tubers of *Curcuma malabarica* (Wilson *et al.*, 2005), *E. coli* and *S. aureus* in *Melia azedarach* (Sen and Batra, 2012), *C. albicans*, *P. aeruginosa*, in leaves of *Catharanthus roseus* (Chaman *et al.*, 2013) *Streptococcus mutans* in and *Mentha piperita* (Abdul *et al.*, 2019), *P. aeruginosa* in *Agrimonia eupatoria* (Ginovyanyan *et al.*, 2017), and *S. typhi*, *E. coli*, *B. megaterium*, *P. aeruginosa* in *Piper nigrum* (Singh *et al.*, 2018).

It was noted that all extracts like methanol extract, ethanol extract, chloroform extract, aqueous extract, n-hexane and acetone extract exhibited antimicrobial activity against all bacterial and fungal strains tested in the present study (Table 2 and 3). The bacterial strains recorded differential sensitivity for each extract. Antimicrobial activity was not found with controls (DMSO and water). Phytochemical constituents of *L. inermis* exhibited antimicrobial activity only against gram-positive bacteria (*S. aureus*, *B. subtilis*) while ineffective for

gram-negative bacteria (*S. typhi*) as noticed by Pappageorgiou *et al.* (2008). Contrary to this, the present study has recorded that the plant extracts have antimicrobial activity against both gram-positive (*S. aureus*, *B. subtilis*) and gram-negative (*E. coli*) bacteria. The work of Habbal *et al.* (2011), Bhuwaneshwari *et al.* (2002) and Hussain *et al.* (2011) also support our outcomes. Habbal *et al.* (2011) found that henna samples demonstrated antibacterial activity against *P. aeruginosa*. Hussain *et al.* (2011) also reported antibacterial activity of *L. inermis* against gram positive (*Bacillus cereus*, *Staphylococcus aureus*, *Corynebacterium bovis*) and gram negative bacteria (*Pasteurella multocida*, *Escherichia coli*). Bhuwaneshwari *et al.* (2002) reported that *L. inermis* had antimicrobial activity against both gram-positive and gram-negative bacteria.

The outcomes revealed that the requirement of the extract was least in the case of *B. cereus* (1.6 and 2.9 mg/ml), and it was high in *P. aeruginosa* (3.6 and 7.1 mg/ml) in both the callus and leaves extracts (Table 4). The range of MIC was lower in the present study as compared to reports of Al-kurashy *et al.* (2011). They found that MIC values were in the range of 8–64 mg/ml for aqueous extract and 32–64 mg/ml for alcoholic extract of *L. inermis* against *E. coli*, *S. aureus*, *P. aeruginosa* and *E. faecalis*.

## Conclusion

*Lawsonia inermis* leaves and callus extracts used in the present study showed antimicrobial activity against the resistant *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*

and *B. cereus* bacterial and *C. albicans* fungal strains. Thus, the study suggests using these plants to treat various ailments caused by pathogenic and nonpathogenic strains of bacteria and fungus. Further, the potential of these plants must be traversed more and more to develop an alternate therapy for the treatment of infections caused by an antibiotic-resistant microorganism.

### Conflict of interest

The authors declare that they have no conflict of interest.

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