Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(3):571-575

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical analysis and antibacterial studies of *Lawsonia inermis* leaves extract

Mohd Yusuf

Department of Chemistry, YMD College, M. D. University, Haryana- 122107, India

---

ABSTRACT

The leaves extracts of Lawsonia inermis, a member of the family Lythraceae, have been extracted by maceration technique in various solvents such as ethanol, ethyl acetate and n-hexane, evaluated for phytochemicals and their antibacterial potential against selected Gram negative (Proteus mirabilis and Pseudomonas aeruginosa) and Gram positive (Staphylococcus epidermidis and Enterococcus faecalis) bacterial isolates. The antibacterial activity was assessed through disc diffusion assay. It has been observed that the extracts of L. inermis leaves, have alkaloids, steroids, flavonoids and terpenoids, and saponins found in ethanol extract only. Glycosides were found in ethyl acetate and n-hexane extracts to a significant extent but glycosides were not recognized in ethanol extract. Maximum antibacterial efficacy was observed in ethanol extract and is most effective and ethyl acetate & n-hexane extracts show considerable antibacterial effect. This study shows that L. inermis leaves contain bioactive phytochemicals that may served as a biocompatible and eco-safe antiseptic or antibacterial agent in the drug formulations.

Keywords: *Lawsonia inermis*, extraction, disc-diffusion assay, antibacterial activity

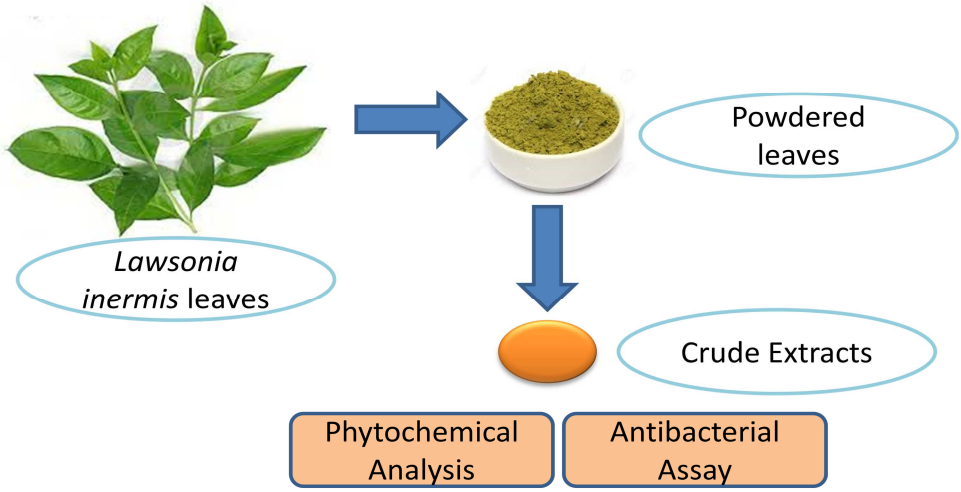
---

INTRODUCTION

Plants have served mankind since its inception. In the present scenario, a great interest has been made towards the natural products derived from medicinal plants all over the world as they show to have enormous health care benefits such as antioxidative, antihypertensive, antimutagenic, bronchodilator, skin disorders, antispasmodic, fever, jaundice, anthelmintic, anti-proliferative, anticancer, antimicrobial, antidiabetic, hepatoprotective, larvicidal, anti-inflammatory, haemolytic activities etc [1-3]. Plants are an important source of bioactive molecules for drug discovery due to the inherent pharmacological activities with low toxicity. India is endowed in the form of medicinal plants having about 45,000 plant species. Recently, around 20,000 medicinal plants have been reported in India, in which a lot of plant species are used since ancient times for curing different diseases [4].

Currently, the infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant ability of some microorganisms. In addition, synthetic antimicrobial agents are often associated with the adverse effects on the host, including immune suppression, hypersensitivity and several allergic responses [5]. This situation reinforced the scientist communities looking for eco-friendly alternatives so that novel bioactive therapeutic agents can be made.

Lawsonia inermis, commonly known as Mehdi/Mehandi is a shrub or small tree frequently cultivated in India, Pakistan, Egypt, Yemen, Iran and Afghanistan. Henna is an ancient dye, evidence being the Egyptian mummies found in the tombs that had their nails dyed with henna. It is also used in many countries for dyeing hair, eyebrows and fingernails during religious festivals and marriages etc. the powdered leaves of this plant (aqueous paste) are used as a cosmetic for staining hands, palms, hairs and other body parts [6-8].



The present paper discusses the comparative suitability of antibacterial potential of *L. inermis* leaves extract as a cleaner alternative to the toxic and unsafe antibacterial agents that is a need for the society of eco-preservation and environmental safety to make a greener world.

EXPERIMENTAL SECTION

Strains and media

Four bacterial strains were selected due to their popularity and suitability. The pure cultures of organisms, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus epidermidis* and *Enterococcus faecalis* (Gram positive) were sub-cultured in McConky agar (HiMedia, India) nutrient broth. They were inoculated, separately, into nutrient broth and kept at 37°C for 24 hours. Then, they were kept at 4°C until further use.

Chemicals and reagents

All the chemicals and reagents in this study were of Laboratory grade and used without further purification.

Plant sample collection and preparation of extracts

Fresh and healthy leaves of *L. inermis* were collected from college garden, Nuh, Haryana. Thereafter the leaves were air-dried in shade at room temperature for 4 weeks and ground with an electric grinder to obtain fine powder. The powdered leaves were stored in a sealed bottle at room temperature.

100 g of powdered sample was transferred into round bottom flask (capacity 1000 mL) fixed with a magnetic stirrer and 750 mL solvents were (Ethanol, Ethyl acetate and *n*-Hexane) added for 72 h. The plant extract was then collected and filtered through Whatman No.1 filter paper. The filtrates were concentrated *in vacuo* using a rotary evaporator at 45 ^{o}C followed by dried in a desicator and crude extracts stored in refrigerator for use.

Phytochemical analysis

The stock solution was prepared from each of the crude extracts (ethanol, ethyl acetate and *n*-hexane). The obtained stock solutions were subjected to phytochemical analysis based on standard methods described [10-12].

Test for alkaloids

15 mg of each extract was separately stirred with 6 mL 1% dil. HCl on a water bath for 5 min and filtered. These filtrates were divided into three equal parts.

(a) *Dragendorff's test*: To one portion of the filtrate, Dragendorff's reagent (Potassium bismuth iodide solution) (1 mL) was added; an orange red precipitate shows the presence of alkaloids.

(b) *Mayer's test*: To one portion of filtrate, Mayer's reagent (Potassium mercuric iodide solution) (1 mL) was added. Formation of cream coloured precipitate gives an indication of the presence of alkaloids.

(c) *Wagner's test*: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the filtrate; a brown coloured precipitate indicates the presence of alkaloids.

Test for steroids
The crude extracts (0.1 gm) were dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall side. The colour of the upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for flavonoids
A few drops of dil. sodium hydroxide solution were added to the stock solution of crude extracts (0.5 ml). An intense yellow colour appeared in the plant crude extract, which became colourless upon the addition of a few drops of dil. H_2SO_4 acid. This shows the presence of flavonoids.

Test for terpenoids
(a) *Salkowski test*: The crude extract (about 100 mg) was separately shaken with chloroform (2 mL) followed by the addition of concentrated H_2SO_4 (2 mL) along the side of the test tube, a reddish brown colouration of the interface indicates the presence of terpenoids.

(b) *Liebermann-Burchard test*: Each extract (100 mg) was shaken with chloroform in a test tube; few drops of acetic anhydride was added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated H_2SO_4 (2 mL) was added along the side of the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids while formation of deep red colour indicates the presence of triterpenoids.

Test for tannins
Extract (100 mg each) was separately stirred with distilled water (5 mL) and then filtered. A few drops of 5% ferric chloride solution then added. Black or blue-green colouration or precipitate was taken as positive result for the presence of tannins.

Test for Saponins
The stock solution from each crude extract (50 mg) was dissolved in distilled water (10 ml) and then the test tube was shaken by hand for 15 min. The formation of a foam layer on the top of the test tube showed the presence of saponins.

Tests for glycosides
(a) *Anthraquinone glycoside (Borntrager's test)*: To the extract solution (1 mL), 5% H_2SO_4 (1 mL) was added. The mixture was boiled in a water bath and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red colour of the ammonia layer gives indication of anthraquinone glycosides.

(b) *Cardiac glycoside (Keller-Killiani test)*: Extract (1 mL) was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed by H_2SO_4 (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive result for cardiac glycoside.

Antibacterial activity assay
Antibacterial assessment was carried out by means of disc diffusion assay method [13,14]. Negative controls were prepared by using the same solvents employed to dissolve the samples. Inhibition zones were measured and compared with Ceftriaxone as standard reference antibacterial agent. Whatman No. 1 sterile filter paper discs (4 mm in diameter) were impregnated with extracts and placed on the inoculated agar. All the plates were incubated at 37 ^oC for 24 h.

The diameter of zone of inhibition was recorded in mm after 48 h, clear zone of inhibition was measured and compared with that of control. The experiment was performed for both the Gram (–ve) & Gram (+ve) bacterial strains.

Index of sensitivity is defined as: Zone diameter (mm)/concentration (μg/mL) = clearing (mm/μg)

Evaluation of antibacterial activity was measured as the diameter of the zones of inhibition against the tested microbes. Each method in this experiment was replicated three times. Values were shown in terms of Mean ± SD error of all three respective categories.

RESULTS AND DISCUSSION

Phytochemical analysis

The leaves extracts (ethanol, ethyl acetate and *n*-hexane) of *L. inermis* have been analyzed for phytochemical contents. From the Table 1 it can be observed that the extracts of *L. inermis* leaves contain phytochemicals namely alkaloids, steroids, flavonoids and terpenoids. Therefore, saponins also found in ethanol extract while ethyl acetate and *n*-hexane extracts do not show saponins. Glycosides were found in ethyl acetate and *n*-hexane extracts to a significant extent but glycosides were not recognized in ethanol extract.

Table 1: Phytochemical analysis of *L. inermis* leaves

Phytochemicals	Ethanol extract	Ethyl acetate extract	*n*-Hexane extract
Alkaloids			
Dragendorff's test	+	+	+
Mayer's test	+	+	+
Wagner's test	+	+	+
Steroids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	-
Salkowski test	+	+	+
Liebermann-Burchard test	+	+	+
Tannins	-	-	-
Saponins	+	-	-
Glycosides	-	-	-
Borntrager's test	-	-	-
Keller-Killiani test	-	+	+

+ = presence and - = absence

Antibacterial activity

In the present study different extracts of *L. inermis* leaves were evaluated for their antibacterial potential against Gram negative and Gram positive bacterial strains using different solvents *viz.* ethanol, ethyl acetate and *n*-hexane. Table 2 represents the zone of inhibition of various extracts with ceftriaxone as a standard reference. From the Table 2 it can be seen that ethanol extract of *L. inermis* leaves is most effective and ethyl acetate & n-hexane extracts show considerable antibacterial effect. The infections caused by *P. aeruginosa* observed multi-drug resistance bacterial species and difficult to treat with conventional antibiotics [15,16]. *Pseudomonas aeruginosa* showed much resistance effect in all cases (extract as well as ceftriaxone). All extracts have remarked antibacterial activity against *Enterococcus faecalis* possessing maximum zone of inhibition. As the leaves extracts exhibited pronounced activity comparable with standard antibacterial agent (ceftriaxone) towards tested microbial isolates, it can be used as an eco-safe, biodegradable alternative in prevention and treatment of bacterial infections.

The variation of the susceptibility of microorganisms towards the *L. inermis* leaves extract could be attributed to the presence of bio-active phytochemicals and their intrinsic properties that are related to the permeability to the cell surface of micro-organisms [13]. Due to the emergence of the antibiotic resistant pathogens, medicinal plants have found the better platform and could be excellent alternates to combat the spread of multi drug resistant micro-organisms [2-5]. Thus, the results from the present study are quite convenient, and *L. inermis* leaves extract would be taking into consideration to the interesting promise of designing a potentially active antibacterial synergized agent of plant origin.

Table 2: Antibacterial activity of various extracts of *L. inermis* leaves

Micro-organisms tested	Zone of inhibition (1000 µg/mL)			Ceftriaxone (1000 µg/mL)
	Ethanol extract	Ethyl acetate extract	*n*-Hexane extract	
Proteus mirabilis	17.21	16.94	14.48	19.23
Pseudomonas aeruginosa	16.18	14.97	14.23	16.91
Staphylococcus epidermidis	19.62	18.29	19.25	21.18
Enterococcus faecalis	22.13	21.96	20.89	28.89

Zone of inhibitions were expressed as mean±SD error (0.5) of three replicates.

CONCLUSION

From the present study it was noticed that *L. inermis* leaves have good antibacterial potential against for both Gram native as well as Gram positive bacterial strains. The antibacterial activity of leaves extract of *L. inermis* was found to be quite satisfactory comparable with the standard antibiotics screened under similar conditions. So, it can be recommended as a green alternative to the synthetic antibacterial agents after clinical trials. Due to the

environmental safety and eco-preservation natural sources surely highlight the needs to continue exploration to the innovative research for bright future.

Acknowledgement
The author's thankfully acknowledgement goes to Principal, YMD College, Nuh, Haryana for providing the financial assistance. The author is also grateful to Prof. W.A. Siddiqui, Head, D/O Applied Sciences and Humanities, Jamia Millia Islamia, New Delhi for extending the biological testing.

REFERENCES

[1] R.A. Halberstein. *Annals Epidemiol.*, **2005**, *15*(9), 686-699.
[2] G. Madhumitha, L.A.M. Sara, B. SenthilKumar, A. Sivaraj. *Asian Pacific J. trop. Med*., **2010**; 3, 788-790.
[3] P. Fortini, P. Di-Marzio, P.M. Guarrera, M. Iorizzi. *J. Ethnopharmacol*., **2016**. doi:10.1016/j.jep.2016.03.010
[4] S. Selvakumar. *J. Chem. Pharmaceut. Res*., **2015**, 7(12), 784-787.
[5] I. Ahmad, Z. Mehmood, F. Mohammad. *J. Ethnopharmacol.*, **1998**, 62(2), 183-193.
[6] F. Mayer, A.H. Cook. The Chemistry of Natural Colouring Matters. Reinhold Publishing Corporation, U.S.A., **1943**, pp. 105.
[7] A.C. Dweck. *Int. J. Cosmet. Sci*., 2002, 24, 287-302.
[8] T. Bechtold. Natural colorants- **quinoid**, naphthoquinoid and anthraquinoid dyes. In: T. Bechtold, R. Mussak (Eds.), Handbook of Natural Colorants. John Wiley & Sons Ltd., UK, **2009**, pp. 153-156.
[9] J.B., Harborne. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall; New York, **1998**.
[10] G.E. Trease, W.C. Evans. Pharmacognosy 11th Edn. Macmillian Publishers, Brailliar Tiridel **1989**.
[11] A. Sofowora. Medicinal Plants and Traditional Medicine in West Arica, John Wily & Sons, New York, **1982**.
[12] N. Joshi, S. Bhatt, S. Dhyani, J. Nain. *Int. J. Curr. Pharm. Res.*, **2013**, 5(2), 144-147.
[13] M. Yusuf, A. Ahmad, M. Shahid, M.I. Khan, S.A. Khan, N. Manzoor, F. Mohammad. *J. Clean. Prod.*, **2012**, 27, 42-50.
[14] M. Yusuf, M. Shahid, M.I. Khan, S.A. Khan, M.A. Khan, F. Mohammad. *J. Saudi Chem. Soc*., **2015**, 19, 64-72.
[15] National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January **1990**-May 1999, issued June 1999. A report from the NNIS System. *Am. J. Infect. Control*, **1999**, 27, 520-32.
[16] B. Abu-Shanab, G.A. Dahood, A.N. Jarrar, K. Adwan. *Turk. J. Biol*., **2004**, 28, 99-102.