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SECONDARY METABOLITES AND ANTIBACTERIAL EFFICACY OF *LAWSONIA INERMIS* LEAF EXTRACTED IN DIFFERENT SOLVENTS AGAINST SOME PATHOGENIC STRAINS

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ABSTRACT

Lawsonia inermis (L.) commonly called Mehandi or Heena its leaves, flowers, seeds, roots are used in traditional medicine. Leaf extract of Lawsonia inermis were assessed for their secondary metabolites and antibacterial activity in different solvents like (methanol, ethanol, chloroform, ethyl acetate and aqueous). The antibacterial activity were tested against five bacterial species including (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri and Lactobacillus plantrum) using agar well diffusion method. Estimation of secondary metabolites revealed the presence of alkaloids, terpenoids, flavonoid, tannins, phenols, sugar and saponins. Methanol extract showed the strongest activity against Shigella flexneri, E.coli and minimum activity observed in aqueous extract. Gram-negative bacteria were most susceptible than gram positive in leaf extract of L.inermis. In present investigation, these results indicate that the antibacterial activity could be attributing to presence of secondary metabolites. Additionally, the study suggested that the Lawsonia inermis leaf could be a good source of antibacterial compounds and may prevent from many infectious diseases.

KEY WORDS

Lawsonia inermis, antibacterial, metabolites, E.coli

INTRODUCTION

Traditional healers have long used plants to prevent or cure infectious diseases. As a large portion of population in the world is dependent on plant-based medicines, particularly in developing world, study of medicinal plants becomes imperative. Plants are rich in a wide variety of secondary metabolites polyphenols, such as tannins, terpenoids, alkaloids, and flavonoids, which have been demonstrated to have in vitro antibacterial properties (González-Lamothe, R., 2009, Habbal O.A.,2007). Also, rising global interest in herbal products makes it compulsory to scrutinize the presence of active ingredients for their proper therapeutic applications. On account of this, special attention has been paid to extracts and biologically active compounds isolated from plant species (Chanda S (2011), Jeyaseelan E C (2012)) with a hope to get new promising drugs to treat these resistant stubborn microorganisms.

Lawsonia inermis (Heena) (Lythraceae) is a perennial plant commonly known as Heena. *Lawsonia* is monotypic genus, represented by *Lawsonia inermis*, native of North Africa and south-west Asia, widely cultivated as an ornamental and dye plant throughout India (Goswami, M. et. al., 2011). Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent (Chetty, K.M., 2008), Chopra R.N. et. al., (1956)). Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants,which provide

health benefits for humans (C.M. Hasler and J.B. Blumberg 1999). In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. Phytochemical accumulate in different parts of the plants, such as in the roots, stems and leaves. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anti-cancer property (Ch. Saidulu, et. al., 2014). Henna is an important source of phytochemicals such as naphthoquinone derivatives, aliphatic components, triterpenes, sterols, phenolic derivatives, coumarins, xanthones, flavonoids, gallic acid, hennotannic acid and mannitol which are effective as immunomodulators and other allied agents (Varghese, K.J. et. al., 2010). In the present study phytochemical analysis and antibacterial activity of *Lawsonia inermis* leaf extracted in different solvents against some pathogenic strains.

2. MATERIAL AND METHODS

2.1 Plant material

The plants were harvested from Ch. Devi Lal Herbal Nature Park-Chuharpur, District Yamunanagar, Haryana and maintained in the Kurukshetra University nursery and identified based on ethnomedical data. The leaves were collected and washed thoroughly with water and air dried under shade and ground using a pestle and mortar. The dried powder was stored in an air-tight bottle at 28°C for further extraction.

2.2. Extraction of plant material

Powdered leaves samples (10 g) were separately macerated in 100 ml of solvents (ethyl acetate, methanol, chloroform, ethanol, aqueous) and incubated at room temperature (37^{o}C) for 2 days with intermittent shaking. The mixtures were then filtered through Whatman filter paper no. 1. The filtrates were collected and heated in water-bath at 48^{0}C to evaporate its liquid content. The residue was dried further overnight in an oven at 37^{0}C in a Concentrated sticky sample were reconstituted quantitatively in DMSO/methanol to obtain the extract solution at known concentration. The extract were preserved at -20^{0}C until use.

2.3 Phytochemical analysis:

The extracts of *Lawsonia inermis* prepared in the present study were screened for phytochemicals including carbohydrates, cardioglycosides, alkaloids, tannins, flavonoids, saponins and sugar by phytochemical analysis as below (Thenmozhi, M. et. al., 2010, Harborne, J.B.,1998).

2.4. Test organisms

Anti-microbial activity of various crude extract samples of *Lawsonia inermis* was evaluated by agar well diffusion method (Bouer, et. al*.,* 1966). Test organisms used in this study were *Lactobacillus plantarum* (MTCC 1407), *Staphylococcus aureus* (MTCC 11949) (Gram positive) and *Pseudomonas aeruginosa (*MTCC 3542*), Shigella flexneri* (MTCC-1457), *Escherichia coli* (MTCC-9721) (Gram negative). The strains were maintained on nutrient agar slants at 4 ^{o}C.

2.5 Antibacterial Activity Assay

The antibacterial activity of *Lawsonia inermis* (leaves) from different solvent extracts (Chloroform, Methanol, Ethyl acetate, Ethanol and Aqueous) was evaluated by agar well diffusion method (Bauer, et. al., 1966). 24 hr old broth cultures of the bacteria were used for the antibacterial assay. Mullar Hinton agar (Himedia, india) was used with different extract of 250 µl each were dispensed into each well using sterile micropipette. The DMSO solvent was served as a negative control and 2% tetracycline (Himedia, india) for bacteria as a positive control. The antibacterial activity was determined by measuring the diameter of zone of inhibition (mm).

3. RESULT AND DISCUSSION

3.1 Phytochemical screening

Phytochemical analysis of the methanol and aqueous extracts of *Lawsonia inermis* leaves were carried out to determine the presence of phytochemicals like alkaloids, tannins, flavonoids, saponins, carbohydrate, sugar and glycosides (Table 1). The result showed the presence of alkaloids, glycosides, sugar, flavonoids and tannins in methanolic extract and alkaloids, glycosides, tannins, sugar and carbohydrate in aqueous extract. We did not find a presence of flavonoids in aqueous extract and saponins in methanolic extract.

Table 1: Phytochemical screening of solvent extracts of *Lawsonia inermis* Linn

Phytochemicals	Test/reagents	*Lawsonia inermis*	
		Methanolic	Aqueous
Alkaloids	Mayer's test	+	+
Saponins	Foam test	-	-
Carbohydrates	Millons test	+	+
Sugars	Molisch's test	+	+
Glycosides and Sterols	Salkowaski test	+	+
Flavonoids	Shinoda test	+	-
Tannins	Ferric chloride test	+	+

The various phytochemical compounds detected from *Lawsonia inermis* were used in medicine and have antibacterial significance (Al.Maqtari, M., 2014, Dahake, P.R., and Kamble, S.I., 2015).

3.2 Antibacterial activity

In present study, various extracts of *Lawsonia inermis* were compared for their antibacterial activity with the standard antibacterial drugs. The result shows that methanol extract showed the strongest activity against *Shigella flexneri*, *E.coli* and aqueous extract does not show activity against *P.aeroginosa* and *L.plantrum* while others shows activity. Among all the extracts methanol extract shows good activity. Gram-negative bacteria were most susceptible than gram positive in leaf extract of *L.inermis.* The zone of inhibition obtained was compared to the standard tetracyclin as given in Table 2, Fig.1.

Table 2: Antibacterial activity of various extracts

Test organism	Leaf extract (250 µl)				
	Methanol	Ethanol	Chloroform	Ethyl acetate	Aqueous
E.coli	25	23	20	19	17
P.aeruginosa	24	21	20	17	NZ
S.flexneri	26	15	22	18	13
S. aureus	23	23	18	16	14
L. plantarum	15	10	17	17	NZ
Tetracyclin	29	25	23	23	24

*NZ-no zone

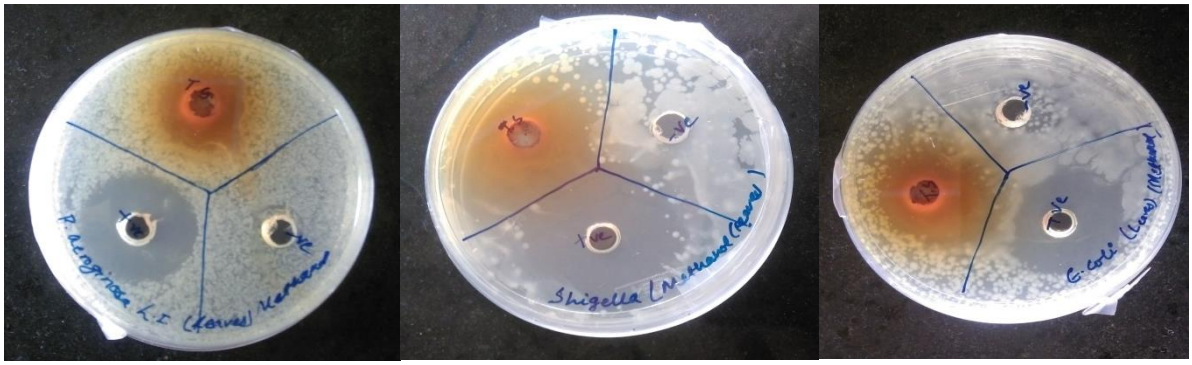

Fig.1 Zone of inhibition (in mm) of methanolic extract of *Lawsonia inermis* leaves against pathogen bacteria

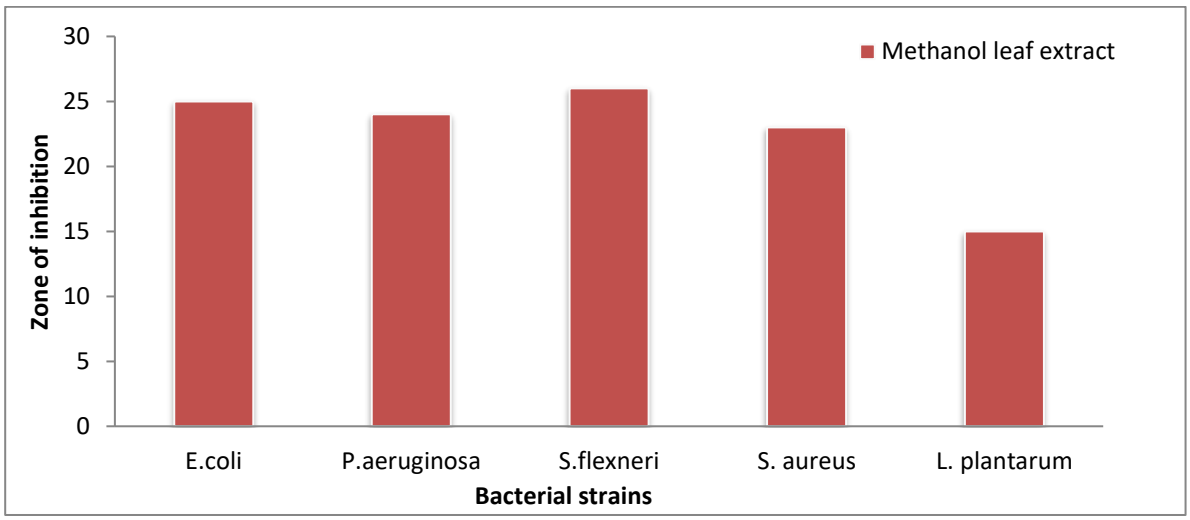

Fig. 2 Anti-bacterial potential of methanolic leaf extract of *Lawsonia inermis against various bacterial strains*

According to the study of Papageorgiou, et. al., (1999), phytochemical constituents of Lawsonia inermis exhibit antimicrobial activity only against gram positive bacteria while ineffective for gram negative bacteria. Other studies have found that *Lawsonia inermis* had antimicrobial activity against both gram positive and gram-negative bacteria (Iram Gull, et al., 2013).

4. CONCLUSION

The phytochemical study of extracts shows the presence of flavonoids, tannins, glycosides, sugar etc. The activity may be due to these compounds. This study shows that the leaves of this plant showed antibacterial activity against *Shigella flexneri* in methanol extract.

5. REFERENCE

Al Maqtari, M., 2014. In vitro Antibacterial activity of Different Yemeni Leaves extracts of *Lawsonia inermis* against Some Bacterial Pathogens. Int. J. Research Stud. in Biosciences. 2, 10;52-57.

Ch. Saidulu., Venkateshwar, C., and Rao, S.G., 2014. Preliminary Phytochemical Studies of Medicinal Plant Drug: *Withania Somnifera* Linn. Biolife. 2, 1;306-312.

Chanda, S., Rakholiya, K., Nair, R., 2011. Antimicrobial activity of *Terminalia catappa L*. leaf extracts against some clinically important pathogenic microbial strains. Chinese Med. 2: 171-177.

Chetty, K.M., 2008. Flowering plants of Chittoor, Edn 1, Andhra Pradesh. 132.

Chopra, R.N., Nayer, S.L., Chopra, I.C., 1956. Glossary of Indian medicinal plants, CSIR Publications, New Delhi. pp. 151.

Dahake, P.R., and Kamble, S.I., 2014. Study on antimicrobial potential and preliminary phytochemical screening of *Lawsonia inermis* Linn. Int. J. Pharm. Sci. Res. Vol. 6(8): 3344-3350.

González-Lamothe, R., Mitchell, G., Gattuso, M., Moussa, S., Malouin, D.F., Bouarab, K., 2009. Plant antimicrobial agents and their effects on plant and human pathogens. Int. J. Mol. Sci. 10(8): 3400-3419.

Goswami, M., Kulshreshtha, M., Rao, C.V., Yadav, S., Yadav, S., 2011. Anti-ulcer potential of *Lawsonia inermis L.* leaves against gastric ulcers in rats. J. App. Pharm. Sci. 01(02): 69-72.

Habbal, O.A., Al-Jabri, A.A., El-Hag, A.G., 2007. Antimicrobial properties of *Lawsonia inermis* (henna): a review. Aust. J. Med. Herbal. 19: 114-125.

Harborne, J.B., 1998. Phytochemical Method 3rd ed.203-214.

Hasler, C.M., and Blumberg, J.B., 1999. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition.vol.129, pp.756S-757S.

Iram, G., Maria, S., Muhammad, S. A., and Muhammad, A. A., 2013. Phytochemical, toxicological and antimicrobial evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. Ann. Clin. Microbiol. Antimicrob. 12:36, 1-6.

Jeyaseelan, E.C., Jenothiny, S., Pathmanathan, M.K., Jeyadevan, J.P., 2012. Antibacterial activity of sequentially extracted organic solvent extracts of fruits, flowers and leaves of *Lawsonia inermis L*. from Jaffna. Asian Pac. J. Trop. Biomed. 2: 798-802.

Papageorgiou, V.P., Assimopoulou, A.N., Couladouros, E.A., Hepworth, D., Nicolaou, K.C., 1999.The chemistry and biology of alkannin, shikonin, and

related naphthazarin natural products. Angew Chem. 38:270–300.

Thenmozhi, M., Sivaraj, R., Hiranmai, Y.R., 2010. A comparative phytochemical analysis of *Alstonia scholaris, Lawsonia inermis, Ervatamia divaricata* and *Asparagus racemosus*. Int. J. Pharma Res. Dev. 2:86–91.

Varghese, K.J., Silvipriya, K.S., Resmi, S., Jolly, C.I., 2010. *Lawsonia inermis* (Henna): A natural dye of various therapeutic uses-A review. Inventi Impact: Cosmeceuticals. ArticleID-Inventi:Cc/3/10.

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