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Phytochemical Screening and Antibacterial Activity of *Lawsonia inermis* Leaf Extract

[1]Wasim Raja, [2]M. Ovais and [3]Amit Dubey

[1]Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, Madhya Pradesh, India
[2]Departments of Biosciences, Barkatullah University, Madhya Pradesh, India
[3]Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh, India

Abstract: The present study is the continuation of a program aimed at investigation of antimicrobial and photochemical properties of *Lawsonia inermis* leaf extract to justify the traditional claim endowed upon this herbal drug as a *rasayana* in Ayurveda. The antimicrobial activity was evaluated according to the disk diffusion method by using Gram positive; *B. subtilius, S. aureus and S. epidermidis* and Gram negative; *E. coli*, *S. flexneri*, *P. aeruginosa* bacteria. This study show that methanolic leaves extracts of *Lawsonia inermis* Linn inhibit the growth of micro organisms dose dependently. Phytochemical screening of the extracts showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids. The presence of flavonoids and glycosides as major constituents of the plant leaves that are commonly known to posses antimicrobial activity. These results confirm the antibacterial activity of *Lawsonia inermis* leaves and support the traditional use of the plant in therapy of bacterial infection.

Key words: Phytochemicals · Antibacterial · Disk Diffusion Method · Bacteria · *Lawsonia inermis*

INTRODUCTION

Some bacteria and fungi are extremely pathogenic causing serious human infections. The discovery of antibiotics to combat these pathogens marked a resolution in the 20th century [1]. Unfortunately, because of the inappropriate use of antibiotics in human and veterinary medicine, certain strains of bacteria and fungi developed the ability to produced substances which block the action of antibiotics or change their target or ability to penetrate cells [2]. Therefore, disease causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Tuberculosis, gonorrhea, malaria and childhood era infections are just a few of the diseases that have become hard to treat with antibiotics. However, a large part of the problem is due to our increasing use and misuse of existing antibiotics in human and veterinary medicine and in agriculture [3]. To substitute synthetic antibiotics, many of today modern and effective drugs have their origin in traditional folk medicine [4, 5]. Therapeutic efficacy of many indigenous plants for many disorders has been described by practitioners of traditional medicine [6-8].

Lawsonia inermis Linn (Henna) is a tropical and subtropical shrub, growing in North Africa, Middle East and Indian subcontinent. The powder made of dried crushed leaves is called henna [9]. When applied in a form of paste onto hair or skin, it imparts a reddish brown coloration lasting for up to twelve weeks. It was used as a hair dye as early as ancient times; for instance, the hair of Egyptian mummies was dyed with henna [10]. Besides its use in cosmetics, henna was also used in Medieval Persian, Arab, Turkish and Jewish medicine to treat headaches [11], skin and teeth diseases, as well as animal bites [12]. In Arab countries, it is still used in folk medicine to treat different skin conditions [13]. Modern pharmacological research on henna and its constituents has confirmed its anti-inflammatory, antipyretic and analgesic effects [2] and discovered its anti carcinogenic

Corresponding Author: Wasim Raja, Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, Madhya Pradesh, India. Tel: +919425447174.

potential [14]. It can also be used to treat pediculosis [15]. The active component of henna is lawsone (2-hydroxy-1,4- naphthoquinone, CAS 83-72-7), which is also the principal dye ingredient. Current research suggests that lawsone is non-problematic for external use because of its low toxicity and genotoxicity [16].

However, very limited data are available about the presence of other additives and contaminants in henna powder, which may adversely affect human health. In certain regions, henna is mixed with minerals containing lead, mercury, copper and zinc in order to strengthen the color [13]. Therefore, we have made this study to evaluate the phytochemical and antibacterial effect of *Lawsonia* inermis leaf extract in test system.

MATERIALS AND METHODS

Plant Material: The identification of the plant *Lawsonia inermis* (family: *Lythraceae*) was done by botanist Dr. S. S. Khan (Voucher Specimen No: WR/102/LGOB/2006), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh (India). The *Lawsonia inermis* (Henna) leaves were collected and dried for few days in shade, which were then powdered and preserved in airtight bottles for further studies.

Extract Preparation: *Lawsonia inermis* L. Leaves (100 g) was defatted with petroleum ether (1000 ml) and the residue was extracted in 50% methanol with the help of separating funnel. The supernatant was collected and concentrated in water bath at 40-50°C and dried in hot air oven at 40°C. The dried powder was kept in air tied box.

Microorganisms: The test organisms included the gram positive bacteria; *Bacillus cereus* (*ATCC 11778)*, *Staphylococcus aureus (ATCC 25923)* and Gram negative bacteria; *Klebsiella pneumoniae (NCIM 2719), Escherichia coli (ATCC 25922)* and *Pseudomonas pseudoalcaligenes (ATCC 17440)*. All the bacterial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Antibacterial Assay: Antibacterial activity of *Lawsonia inermis* L. leaf extract was determined by agar disk diffusion method [17] at four different concentrations i.e., 100, 75, 50 and 25 mg/ml. Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate micro organisms (*Bacillus cereus*, *Staphylococcus aureus* and *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas pseudoalcaligenes*). Discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and sterilized. The discs were then impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (TE- Tetracycline, OF- Ofloxacin, AZ- Azithromycin and PC- Piperacillin) and Gram negative (Fu - Nitrofurantoin, GM - Gentamicin, CX - Cefotaxime and NF –Norfloxaci, 5 µg/disc) bacteria were used as standard. The plates were incubated at 37°C for 24 hrs and the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their confirmation.

Preliminary Phytochemical Screening: Phytochemical screening was carried out for henna leaf samples using the method adopted by Crombie *et al*. [18]. Photochemical screening tests of methanolic extracts were carried out for leaf of *Lawsonia inermis* constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins using standard procedures [19, 20].

RESULTS

The results confirmed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids in extracts of the plant. These phytochemical constituents are good source of antimicrobial and antioxidant activity [21]. Phytochemical analysis results of *Lawsonia inermis* leaves were reported in Table 1.

The results showed that the extracts of *Lawsonia inermis* L. has a concentration dependent antibacterial activity with more sensitivity for Gram negative bacteria than Gram positive bacteria used in the study. The extracts of *Lawsonia inermis* showed considerable antibacterial activity at all the four concentrations 100, 75, 50 and 25mg/ml (Table 2).

Table 3 shows the sensitivity of the tested bacteria to the standard antibiotics.

DISCUSSION

Lawsonia inermis has many traditional and commercial uses, the most common being as a dye for hair, skin and fingernails, as a dye and preservative for leather and cloth and as an antifungal. Medicinal plants have provided copious leads to combat diseases, from the dawn of civilization. The extensive survey of literature

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

S.No.	Tests	Tests/Reagents	Level*
1	Glycosides	Borntrager's	+
2	Phytosterol	chloroform	+
3	Steroidal compounds	*Salkowski's Test*	+
		Lieberman's Test	+
4	Saponins	Froth test	_
5	Tennins	Ferric chloride test	+
		Formaldehyde test	+
		Test for phlobatanins	+
6	Flavonoids	Test for free flavonoids	+
		Lead Acetate Test	+
		Sodium Hydroxide	+

*Here, + :presence, - :absence,

Table 2: Antimicrobial activity of *Lawsonia inermis* Leaves extract against tested bacteria

	Name of the Microorganism (Inhibition zone in mm)				
	Gram Positive		Gram Negative		
Test sample concentration in (mg/ml)	*Bacillus cereus*	*Staphylococcus aureus*	*E. coli*	*Kleb. pneumoniae*	*Ps. pseudoalcaligenes*
100	14.0	9.9	16.0	10.2	10.0
50	12.0	8.5	9.0	9.5	8.5
75	9.0	8.0	8.5	9.0	8.0
25	8.0	7.5	7.0	7.0	6.0

Table 3: Sensitivity of the tested bacteria to the standard antibiotics

			Zone of Inhibition (mm)			
			Different concentrations of Antibiotic (5µg/disc)			
S.NO.	Gram Positive/ Negative	Organisms	TE	OF	AZ	PC
1	Gram Positive	*B. cereus*	14	16	18	14
		S. aureus	15	16	16	14
			Fu	GM	CX	NF
2	Gram Negative	*E. coli*	12	16	8	16
		Kleb. pneumoniae	14	13	18	20
		Ps. pseudoalcaligenes	18	18	12	21

TE = Tetracycline, OF = Ofloxacin, AZ = Azithromycin, PC = Piperacillin Fu = Nitrofurantoin, GM = Gentamicin, CX = Cefotaxime, NF =Norfloxacin

revealed that *L. inermis* L. is highly regarded as a universal panacea in the herbal medicine with diverse pharmacological activity spectrum. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible of the various activities of the plant. Hence extensive investigation is needed to exploit their therapeutic utility to combat diseases. A drug development programme should be undertaken to develop modern drugs with the compounds isolated from henna [22].

The present study was conducted to study the antibacterial activity of *Lawsonia inermis* used by Indian peoples to show that therapeutic properties. The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent. Six bacteria's were used for antibacterial studies. Medicinal plants are being used by large proportion of Indian population. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants [23].

In the present study, the results were encouraging, as the *Lawsonia inermis* appeared to contain substances that have antimicrobial properties. The methanolic extracts of *lawsonia inermis* leaves were active against six different bacteria's. Four concentrations of the extract were used (100, 75, 50 and 25 mg/ml). It was estimated that if an inhibition is obtained by 25-100 mg/ml) of test solution, the extract can be considered worthy for further investigations. The Phytochemical study confirmed the presence of glycosides, phytosterol, steroids, saponins,

tannins and flavonoids in extracts of the plant. These phytochemical constituents are good source of antimicrobial and antioxidant activity [21].

Plant methanolic extract showed significant antibacterial activity as compared to standard antibiotics. . That activity might be due to the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids.. Among the various microorganisms, the methanolic extract of L*awsonia inermis* was more active against *E. coli*. Further evaluation needs to be carried out on *L. inermis* L. in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

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