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In vitro Evaluation of Phytochemicals and Antimicrobial activities of extracts of seeds and leaves of *Lawsonia inermis* Linn.

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ABSTRACT

In the last two decades, antibiotic resistance has become a severe problem worldwide. This has lead to the search for new, safe and effective antimicrobial agents from alternative natural resources like plant products. Different parts of *Lawsonia inermis* L. (Family: Lythraceae) like roots, bark, leaves, flowers and seeds are traditionally used for the treatment of various ailments. Alkaloids, tannin, phenolics, saponin and anthraquinone are present in both types of extracts of leaves of *Lawsonia inermis.* Glycosides are present in ethanolic extract of leaves and seeds. Terpenoids and flavonoids are not found in leaves but present in seeds. The disc diffusion assay showed that the leaves and seeds of Henna plant have different degrees of bacterial and fungal growth inhibition, depending on the strains. The aqueous extracts showed broadest antimicrobial activity by inhibiting more or less most of the microbial strains involved. Aqueous extract was more effective than their ethanolic extracts. *Klebsiella pneumonie and Serratia marcescens* were the most resistant strains tested. It could be concluded that seeds and leaves of *Lawsonia inermis* have potential antimicrobial efficacy. All extracts (aqueous or ethanolic) have shown better antimicrobial efficacy against *Staphylococcus aureus, Escherichia coli and Aspergillus niger*. Differential antimicrobial activity of plant parts against different bacteria might be due to concentration of different active phyto-compounds in different parts. Among those antimicrobial compounds, phenolic compounds, terpenoids, and alkaloids are very important compounds in antimicrobial or antioxidant action.

Keywords: Henna, *Lawsonia inermis*, Antimicrobial, Phytochemical

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INTRODUCTION

In the last two decades, antibiotic resistance is emerging as a serious problem worldwide [1, 2]. This has lead to the exploration for new, safe and effective antimicrobial agents from alternative natural resources like plant products. At the same time, there is a growing demand among consumers for natural preservative or additives in processed foods [3]. In comparison to chemical or synthetic additives herbal preservatives are preferred as these are cheaper and without any side effects [4]. Herbal extracts are fast becoming popular as natural antimicrobial or preservatives [5-7]. Traditionally the people of India have a long-standing practice of using extensive diversity of plant products in treatment of diseases. Plants traditionally used for medicinal purpose in different parts of the world have been screened for possible antimicrobial action by several workers [8,9]. Antibacterial activities of extracts of different plants against various microorganisms have been reported by many scientists [10-14]. Some medicinal herbs have also been assessed [8, 15-17]. *Lawsonia inermis* L. (Family: Lythraceae) commonly known as henna is a biennial dicotyledonous shrub with red flowers. It widely occurs specially in drier parts of India and also in Africa and South-West Asia [18, 19]. Henna is a glabrous much branched deciduous shrub with small, simple, opposite, entire, lanceolate leaves; flowers white coloured in cyme; fruits globose, capsules with numerous brown and pyramidal seeds [18, 20]. Different parts of this plant like roots, bark, leaves, flowers and seeds are traditionally used for the treatment of various ailments [19]. Leaves are useful for the treatment of headache, arthritis, diarrhea, dysentery, leprosy, scabies and boils. Flowers are used in burning sensation, anemia, insomnia and fever[20, 21]. Seeds are effective in the treatment of intermittent fever, insanity, dysentery, diarrhea, and amentia[22]. The bark is given for treatment of enlargement of spleen and persistent skin diseases and the root is regarded as a remedy for leprosy[19]. There are several reports about free radical scavenging and reducing power[23], anti ulcer potential [24], hypoglycemic and antihyperglycemic effect[25] and anthelmintic activity[26] by whole plants or by leaves. Leaves extracts showed antifungal activity[27, 28] and antibacterial activity [18, 19, 29, 30]. This study was carried out to evaluate the *in vitro* antibacterial activity of extracts of seeds and leaves of local *Lawsonia inermis* L. against some selected bacteria.

MATERIALS AND METHODS

Plant materials

Fresh and healthy seeds and leaves of *Lawsonia inermis* L. of family Lythraceae were collected in May, 2013, from town of Adra (approximately 86°67 E and 23°49 N) in Purulia District, West

Bengal, India. The plant specimen was indentified in Plant Taxonomy Section, Department of Botany, University of Kalyani. The parts were separately cleaned thoroughly to make those completely free from any possible contamination. The parts were dried in hot air oven at 55°C for 5-6 days, then separately ground into fine powder using a mechanical grinder and sieved. These powder samples were kept in dark coloured glass bottles and subsequently used.

Preparation of aqueous extract

Aqueous extract of each part was prepared by boiling 20 g of dried powder in 100ml sterile distilled water over moderate flame for 20 min. The aqueous extract was cooled, double filtered through Whatman No.1 filter paper and then kept in sterile screw capped glass vials at 4ºC. The aqueous extracts were re-confirmed as free of any contamination by plating method [31]. These crude aqueous extracts were diluted with sterile double distilled water (which is to be used as negative control) to obtain required concentrations before experiments.

Preparation of solvent extraction

Ten gram of each powder sample was crushed in ethanol for 48 hours at 24ºC with stirring [32]. The extracts were centrifuged and filtered through Whatman No.1 filter paper and evaporated using vacuum rotary evaporator to near dryness and stored in glass vials in dark at 4ºC. These crude solvent extracts were diluted with 10% dimethyl sulphoxide (DMSO- which is to be used as negative control) to obtain required concentration before experiments.

Test micro-organisms

Four enteropathogenic, three food-spoiler and one probiotic bacterial strains were selected for the antimicrobial study of the extracts. The strains selected were *Salmonella enterica* serovar typhimurium MTCC 3224, *Serratia marcescens* MTCC 4822, *Escherichia coli* MTCC 3221, *Klebsiella pneumoniae* subsp pneumoniae MTCC 6644, *Proteus vulgaris* MTCC 7299, *Staphylococcus aureus* MTCC 7405, *Bacillus cereus* MTCC 6909, *Lactobacillus brevis* MTCC 4460 were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial cultures were maintained on tryptic soy agar (HiMedia) and subcultured regularly. The fungal strain *Aspergillus niger* was taken from laboratory collection (isolated from bread) and grown on Sabouraud dextrose agar (HiMedia). Standard inocula were prepared by sub-culturing 4-5 freshly grown isolated colonies of each strain in Tryptic soy broth (TSB) and incubated at 35-37 ºC for 24 hours. Inocula were standardized with sterile TSB to give final cell load of 10^6-10^7 CFU/ml.

Phytochemical evaluations

The extracts were tested to phytochemical evaluation using standard techniques of plant secondary metabolites according to Harborne and Turner[33], Evans[34] and Thenmozhi et al[35].

Extracts were tested for phenolics, tannin, flavonoids, alkaloids, triterpenoids, saponin, steroids, coumarin, anthraquinone and glycosides.

Disc diffusion bioassay

The disc diffusion experiment was carried out as described by Jorgensen *et al.*[36]. A 0.4 ml standardized inoculum suspension of each bacterial strain was spread on TSA plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no.1 filter paper discs were aseptically placed on plates. Extracts of standard concentrations (10 mg dry weight) were aseptically poured on the discs along with sterile double distilled water or 10% DMSO as negative and ampicillin as positive controls. Plates were allowed to stand for 30 minutes at room temperature prior to incubation at 35-37 ºC for 24 hours. The inhibition zone diameters were measured at least three times and means were represented.

Determination of MICs

Minimum inhibitory concentrations (MICs) were determined by broth dilution method in culture tubes [36]. Various concentrations (50, 40, 30, 25, 20, 15, 10, 7.5, 5, 2.5, 1.25 mg dry weight/ml) of the extracts were added to broth immediately after inoculating with fresh 0.2 ml culture of the strain, keeping final volume at 5 ml. The cultures were incubated on a rotary shaking incubator at 37ºC for 48 hours. The lowest concentration of the spice or herbal extracts showing no visible growth was considered as the MIC.

Statistical analyses

The experiments were done at least twice and their mean values were represented. All statistical analyses including ANOVA were done in SPSS Version 17.0. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Tannin, phenolics, saponin and anthraquinone are present in both types of extracts of seeds and leaves of *Lawsonia inermis* (Table 1).

Alkaloids are present in extracts of leaves but absent in seeds. Glycosides are present in ethanolic extract of leaves and seeds. Flavonoids are not found in leaves extracts but present in seeds. Tannin, phenolics, flavonoids and saponin are present in both types of extracts of seeds of *Lawsonia inermis* while glycosides and terpenoids are present in only ethanolic extract of seeds. Saponin was not found in study of Raja *et al.* [37].

Table 1: Phytochemical qualitative evaluation of extracts of seeds & leaves of *Lawsonia inermis* L.

	Leaves		Seeds	
	Aqueous	Ethanolic	Aqueous	Ethanolic
Alkaloids	+	+	-	-
Tannin	+	+	+	+
Phenolics	+	+	+	+
Flavonoids	-	-	+	+
Saponin	+	+	+	+
Glycosides	-	+	-	+
Terpenoids	-	-	-	+
Anthraquinone	+	+	+	+

The disc diffusion assay showed that these parts of Henna plant have different degrees of bacterial and fungal growth inhibition, depending on the strains (Table 2).

Table 2: Antibacterial activities, indicated by diameter of inhibition zone (DIZ, mm, for 10 mg dry wt./ disc, Mean±SD) of different extracts of *Lawsonia inermis* L. against the micro-organisms [- means <7mm DIZ i.e DIZ of negative control]

Microorganisms	Leaves		Seeds	
	Aqueous	Ethanolic	Aqueous	Ethanolic
E.coli	11±1	8±0.577	11	12
S. aureus	13±1.527	10±0.577	10	8
S. enterica	8±1	-	12	13
S. marcescens	-	-	8	-
K. pneumoniae	-	-	-	-
P.vulgaris	9±0.577	-	10	-
B. cereus	12±1	8±1.527	9	9
L.brevis	11±1.527	9±0.577	8	8
A.niger	12±1.527	8±1	9	11

The aqueous extracts showed broadest antimicrobial activity by inhibiting more or less most of the microbial strains involved. Aqueous extract was more effective than their ethanolic extracts. Widest inhibition zones (12-13 mm DIZ) were seen in cases of aqueous extracts of henna against *S. aureus, B. cereus* and fungus *A.niger*. Gram positive bacteria were more prone to these herbal extracts than Gram negative bacteria. *Staphylococcus aureus* was found to be the most sensitive strain. *Klebsiella pneumonie* and *Serratia marcescens* were the most resistant strain tested. Raveesha et al. reported antifungal activity of *Lawsonia inermis* against seed- borne pathogen of *Aspergillus sp* [38]. Extracts of seeds are more effective against *Salmonella enterica*. It is clear that different extracts or decoctions of herbs differ in their anti-microbial activities, which may depend on solubility of the active constituents. The antimicrobial activities of plant extracts are dependent on various factors like the environmental and climate conditions under which the plant grew, the solvent used for the extraction, the extraction method, test concentration and the test microorganisms [19].

The MIC assay of aqueous and ethanolic extracts showed that henna had very good anti-microbial action against the strains tested except 2-3 strains (Table 3). Whether the aqueous or ethanolic extract would work well on microbes, it all depends on active constituents [39]. The principal antimicrobial chemical constituents of henna are lawsone, 2-hydroxy-1:4 napthaquinone ($C_{10}H_6O_3$), gallic acid, hennatannic acid, resins etc. [18]. Among the microbial strains tested, *Klebsiella pneumonie* and *Aspergillus niger* were the most resistant microbes while *Staphylococcus aureus* and *E. coli* were the most susceptible strains. Henna worked very well against *E. coli* though *E. coli* was earlier shown as resistant to different anti-microbial agents [40].

Table 3: Antibacterial activities, indicated by minimum inhibitory concentrations (MIC, mg dry wt. ml^{-1}) of different extracts of *Lawsonia inermis* L. against the micro-organisms

Microorganisms	Leaves		Seeds	
	Aqueous	Ethanolic	Aqueous	Ethanolic
E.coli	10	30	10	10
S. aureus	10	30	15	50
S. enterica	30	50	30	10
S. marcescens	50	>50	30	30
K. pneumoniae	50	>50	>50	>50
P.vulgaris	30	30	30	>50
B. cereus	10	30	>50	50
L.brevis	15	40	50	50
A.niger	10	30	30	15

CONCLUSION

It could be concluded that seeds and leaves of *Lawsonia inermis* have potential antimicrobial efficacy. All extracts (aqueous or ethanolic) have more or less antimicrobial efficacy against the microbes examined. Differential antimicrobial activity of plant parts against different bacteria might be due to concentration of different active phyto-compounds in different parts. Among those antimicrobial compounds, phenolic compounds, terpenoids, and alkaloids are very important compounds in antimicrobial or antioxidant effects [41-43]. Further study is required to determine the different antibacterial compounds from this very promising plant and their full spectrum of efficacy.

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