



RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF LAWSONIA INERMIS AGAINST HUMAN PATHOGENIC BACTERIA

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Abstract

Natural medicine has been the main source of primary healthcare all over the world. Since ancient times, plants have been used as a rich source of effective and safe medicines. *Lawsonia inermis* has efficacy on skin disease. Leaves of *Lawsonia inermis* provide an important cosmetic dye. Henna leaves were extensively used for centuries in the Middle East, the Far East and Northern Africa as dye for nails, hands, hair and textile. *Lawsonia inermis* contained 2-hydroxyl-1,4-naphthoquinone (lawsone). Properties of *Lawsonia inermis* include antimicrobial, antifungal, antitumor, antiangiogenic, larvicidal, antileishmanial, antimalarial, hepatoprotective, anti-inflammatory, analgesic, and antipyretic characteristics. In this study the antibacterial activity of the leaf extract of *Lawsonia inermis* on *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. Zone of Inhibition was observed. *Lawsonia inermis* leaves extract containing test disk inhibited growth of pathogenic bacteria. Test disk containing 250 µg, 500 µg and 1000 µg showed 11mm, 14mm and 18 mm zone of inhibition against *E. coli*. 8 mm, 11mm and 13 mm zone of inhibition against *Staphylococcus aureus*. 7 mm, 9mm and 12 mm zone of inhibition against *Streptococcus pyogenes*. Whereas 30 µg Amikacin containing standard antibiotic disk showed 14 mm, 12 mm and 10 mm zone of inhibition against *E. coli*, *S. aureus* and *S. pyogenes*. This research concluded that *Lawsonia inermis* leaves extract contain antimicrobial properties. The formulation of *Lawsonia inermis* leaves extract as a natural source for the development of drugs.

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Introduction:-

Since the beginning of civilization, medicinal plants have been an integral element of human society in the fight against disease. Pathogens continue to exhibit alarming levels of resistance to such antimicrobial drugs worldwide.^[1] The widespread use of antibiotics has led to an increase in antibiotic resistance, which may make some bacterial illnesses more difficult to treat with current antimicrobials.^[2] Therefore, steps must be taken to decrease bacteria resistance, and other antibiotic sources must be investigated.

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[3] Products are utilized in the pharmaceutical business in their purified form or in their natural forms in conventional herbal remedies. [4] The tropical and subtropical shrub *Lawsonia inermis* Linn, sometimes known as henna, is found in North Africa, the Middle East, and the Indian subcontinent. Henna is the name of the dried, ground-up leaf powder. [5] *Lawsonia inermis* Linn. (Family: Lythraceae) is a tiny tree or shrub with many branches that grows to a height of 2 to 6 meters. It is grown mostly for its leaves, however traditional medicine has also made use of the stem bark, roots, flowers, and seeds. This plant is widely used as a cosmetic to dye nails, skin, and hair. [6] *Lawsonia inermis* exhibited a wide range of pharmacological effects, including those that were antibacterial, antifungal, antiparasitic, antioxidant, hepatoprotective, analgesic, anti-inflammatory, antipyretic, wound healing, immunomodulatory, antidiabetic, hypolipidemic, antiulcer, antidiarrheal, diuretic, and anticancer. [7]

Materials and Methods:-

Plants extraction preparation

The leaves of *Lawsonia inermis* plant used in this study were obtained from the campus of Hamdard University in Bangladesh. The leaves were thoroughly cleaned and rinsed with distilled water before being dried in the shade. The dried leaves of *Lawsonia inermis* were grounded into fine powder to pass 100 mm sieve. 100 g of the fine powder was soaked in 400 ml of methanol with stirring for 72 hours, filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (1) to attain a clear filtration. The filtrate was evaporated and dried at 45°C temperature, using water bath. The extract yield was weighted, stored in small bottle in fridge at 5°C and their yield percentages were calculated using the following formula: Extract yield% = $R/S \times 100$ (Where R; weight of extracted plant residues and S; weight of plant raw sample).

Antibacterial activity of the plant extracts

E. Coli, *Staphylococcus aureus* and *Streptococcus pyogenes* were provided from the diagnostic centers of Hamdard General Hospital, Gazaria, Munshigonj. Mueller Hinton agar media and blank disk was purchased from Tradesworth Ltd. & Technoworth Associates Ltd. Dhaka, Bangladesh.

The inoculum of bacterial stain was prepared by sub-culture of each bacterial stain at the temperature of 35°C incubated overnight in Mueller-Hinton agar SLANTS. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10^7 CFU/ml using spectrophotometer.

The disk diffusion method is used to evaluate antimicrobial activity of *Lawsonia inermis* leaves extract. The plant extract residues (50 mg) were re-dissolved in 2.5 ml of ethanol, sterilized through Millipore filter (0.22 µm) then loaded over sterile blank disks to obtain final concentration of 10 mg/disc. 10 ml of Mueller-Hinton agar medium was poured into sterile petri dishes followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) to attain 105 CFU/ml of medium. Sterile blank discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hinton agar plates. Blank disk loaded with 30 µg of Amikacin was used as positive control. The plates were kept in the fridge at 5°C for 2 hours to permit plant extracts diffusion then incubated at 35°C for 24 h. The presence of inhibition zones was measured by Vernier caliper, recorded, and considered as indication for antibacterial activity.

Result:-

Table 1: Zone of inhibition produced by methanolic extract of *Lawsonia inermis* (MELI) leaves extract 250 µg, 500 µg and 1000 µg containing paper disk and 30 µg Amikacin containing antibiotic disk against *E. coli*, *S. aureus* and *S. pyogenes*.

Sample Name	Concentration	Zone of inhibition (mm)		
		<i>E. coli</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
MELI	250 µg	11	8	7
MELI	500 µg	14	11	9
MELI	1000 µg	18	13	12
Amikacin	30 µg	14	12	10

Note: MELI (Methanolic extract of *Lawsonia inermis*)

Discussion:-

Raja et al., (2013) showed their study that methanolic leaves extracts of *Lawsonia inermis* Linn inhibit the growth of

microorganism dose dependently.[8] In this study *Lawsonia inermis* leaves extract used for the antimicrobial activities assessed by disk diffusion method. The result showed good zone of inhibition by against *E. coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*.

Conclusion:-

The result of this experiment suggest that Methanolic extract of *Lawsonia inermis* leaves have antimicrobial capacity to pathogenic bacteria. *Lawsonia inermis* is a good source of natural antibacterial material.

Conflicts of interests

No conflicting interests are stated by the authors.

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