

***In vitro* Antibacterial and Antifungal Activities of the Saudi *Lawsonia inermis* Extracts Against Some Nosocomial Infection Pathogens**

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The increasing prevalence of antibiotic-resistant pathogens has drawn the attention of the researchers in the pharmaceutical field towards studies on the potential antimicrobial activity of plant-derived substances. This study was conducted to investigate the antibacterial and antifungal activity of leaf extracts of *Lawsonia inermis* (Henna) on some nosocomial infection pathogens. Leaf samples of the plant were collected from Yanbu city, Saudi Arabia. Methanol, ethanol, and hexane extracts were obtained by maceration method. The pathogens were isolated from the hospital environment and identified based on cultural, morphological and biochemical characteristics as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and the yeast *Candida albicans*. The methanol extract was shown high antibacterial and antifungal activity compared with Hexane and Chloroform extracts. Methanol extract expressed highest and broad-spectrum antibacterial activity against pathogenic strains. It exhibited high activity against Gram-positive and Gram-negative bacteria and fungi. The results of this study support the use of *Lawsonia inermis* plant in traditional medicine to treat bacterial and fungal diseases and this plant could be a source of new antibiotic compounds.

Key words: *Lawsonia inermis*; Henna; Antibacterial; Antifungal; Human pathogens; Nosocomial infections.

The increasing prevalence of antibiotic-resistant pathogens may decrease the efficacy of existing antibiotics, making the treatment of patients costly and difficult¹. The emergence of resistant microorganisms is a result of the misuse and overuse of antibiotics and the use of antimicrobial compounds in food animal production to improve animal health and higher production². In 2004, more than 70% of pathogenic bacteria were estimated to be resistant to at least one of the currently available antibiotics³. Methicillin resistant *S. aureus* (MRSA) strains are the most common cause of nosocomial infections and cause high mortality and morbidity rate⁴. Enterococcal infections have emerged as one

of the most common nosocomial infections in the past decade. They are an important cause of infection in organ transplant recipients and other seriously ill patients. Members of these genus, are resistant to a large number of antimicrobial agents⁵. There are also other examples of Gram-negative pathogens: *Klebsiella*, *Escherichia*, and *Pseudomonas*. More than 60% of sepsis cases in hospitals are caused by Gram-negative bacteria². Among them, *Pseudomonas aeruginosa* accounts for almost 80% of these opportunistic infections. Furthermore, approximately 40% of death from nosocomial infections are caused by fungi, and 80% of these are caused by *Candida* and *Aspergillus*⁶. Therefore, there is a continuous and urgent need to explore new antibiotics to overcome the emerging resistance to currently available antibiotics. The herbal medicine still plays a vital role to treat

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infectious diseases in developing countries. Among the known plant species on earth (estimated at 250,000–500,000) only a small fraction have been investigated for the presence of antimicrobial compounds and only 1–10% of plants are used by humans⁷. In the past, plants have been used in traditional herbal medicine. The antimicrobial activity of several plant-derived compounds has been previously reported^{8,9}, and a wide range of active components have been identified¹⁰. Several studies revealed that bioactive compounds from some medicinal plants exhibit anti-carcinogenic, antitumor, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory effects, as well as antioxidant properties¹¹. Henna (*Lawsonia inermis*) the 2-6 m height flowering plant, is the unique species in the genus *Lawsonia* in the family Lythraceae¹². Henna plant is used in traditional medicine to treat a variety of diseases such as a headache, rheumatoid arthritis, ulcers, diarrhea, leprosy, fever, diabetes, and cardiac disease¹³. In addition, this plant is used for controlling infectious diseases. The inhibitory activity of the plant against gram negative, gram positive bacteria, and fungi was described^{14,12}. This activity is attributed to the presence of anthraquinones (lawsone) (2 hydroxynaphthoquinones) as major components of the plant, representing about 0.5-1.5% of henna¹⁵. Lawson is a natural dye used in the Middle East, Saudi Arabia, Asia and parts of Africa to color hand, finger, nails, and hair, having a wide spectrum of biological activities, such as anti-fungal and antibacterial for the skin and hair, antitumor, antibacterial, anti-inflammatory, anti-parasitic, cytotoxic activities. Many reports cite the antibacterial effects of henna plant especially for gram positive bacteria and antifungal activity against dermatophytes, and wound healing^{16, 17, 18}, however few researches described its effect on nosocomial pathogens. Accordingly, this study reports the evaluation of the antagonistic potential of Saudi Henna plant against pathogenic microorganisms contributing to nosocomial infections.

MATERIALS AND METHODS

Plant material

Henna (*Lawsonia inermis* Linn) leaves were harvested from different regions of Yanbu

city, Saudi Arabia in April 2016. The plant material was thoroughly washed with distilled water, dried in the shade and grinded to powder. The powder was stored in airtight containers at room temperature in the dark until use.

Chemicals and culture media

Gentamicin and nystatin were purchased from Himedia (India). The solvents ethanol, methanol, and n-hexane and all culture media were obtained from Merck (Germany).

Preparation of *L. inermis* extracts

The powdered samples of henna leaves were subjected to extraction according to the method of Gupta *et al.* (2009)¹⁹. Briefly, 150 g of air dried powder was soaked in 300 ml of ethanol, methanol, and n-hexane. The mixtures were stirred for 3 days at room temperature, then filtered through Whatman filter No 1. The filtrates were dried and concentrated using Rotary evaporator at 40°C. The crude extracts were weighted and stock solutions were prepared to obtain a final concentration of 300 mg/ml. The extracts kept in the dark at 4°C until use.

Isolation of pathogenic microorganisms

Microorganisms used in this study (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and the yeast *Candida albicans*) were isolated from the hospital environment (staff nurses hands, sinks, tubs, ground (floors), walls, beds, blankets, doors, doors handle, nurse tables, chairs, electronic equipment's, etc) at some hospitals in Yanbu city, Saudi Arabia. Strains were identified using standard microbiological procedures using Bergey's Manual of Determinative Bacteriology²⁰. These nosocomial pathogens were identified by colony characteristics, microscopic morphology, catalase and oxidase reactions, API20E test).

Antimicrobial Activity Assay (Disc Diffusion method)

The antibacterial and antifungal activities of ethanol, methanol and hexane extracts of henna plant were evaluated by disc diffusion method (Kirby and Bauer, 1966). Test microorganisms suspensions (prepared in sterile saline), with a turbidity equivalent to that of 0.5 McFarland standard, were uniformly seeded with sterile swabs onto Muller Hinton Agar (MHA) for bacteria and

Sabouraud dextrose agar (SDA) for yeast. The filter paper discs (6 mm) were impregnated with 20 μ l of the plant extracts (100 mg/ml), dried and carefully laid on the surface of the agar plates inoculated with test microorganisms. The plates were left at 4 °C for 12-16 hours then incubated overnight at 37°C. Inhibition zone of test microorganisms around the paper disks was measured. Disks with ethanol, methanol and hexane solvents were used as a negative control. Antibacterial disks with Gentamicin (10 μ g/disc) and nystatin (100 μ g/disk) were used as positive control. All assays were carried out in duplicate.

Minimum Inhibitory Concentration (MIC) Determination

The MIC of the methanol extract against tested microorganisms was determined by the dilution method. Serial two-fold dilutions in Nutrient broth for bacteria and Sabouraud dextrose broth for yeast (300 mg/ml - 1.56 mg/ml) were prepared from stock solution of the extract. The tubes were then inoculated with 50 μ l of microorganism cultures. Un-inoculated tubes containing growth medium and extract were used as negative controls. The tubes were then incubated overnight at appropriate temperatures. The MIC was defined as the lowest concentration that completely inhibited the growth of the organism.

RESULTS

Lawsonia inermis plant was used to evaluate their antimicrobial activity against eight nosocomial infection pathogens. Three solvents ethanol, methanol, and n-hexane were used for extraction of active compounds. The isolated

pathogens were identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and the yeast *Candida albicans* based on cultural, morphological and biochemical characteristics. Data on antimicrobial activity measured as a zone of inhibition of solvent extracts on test microorganisms are shown in tables 1 and represented by the figure 1. In the present study, the strong in vitro inhibition against isolated microorganisms was recorded in methanol extract of henna with inhibition zone of 30 mm against *S. aureus* (table 1 & figure 1). Also strong inhibitions were recorded with methanol extract against *C. albicans* (26mm), *E. cloacae* (23mm), *E. faecalis* (20 mm) and *K. pneumonia* (19 mm). Ethanol extract showed moderate activity against *S. aureus* and *K.pneumonia* while there was no activity in hexane extract.

Significant antibacterial and antifungal effects were expressed as MIC of methanol extract against tested microorganisms are shown in table 2. The strongest activity of methanol extract was against *S. aureus* with MIC value of 1.5 mg/ml followed by *C. albicans* (3.12 mg/ml).

DISCUSSION

Henna plant has been used over centuries in traditional herbal medicine and as preservative, and cosmetic in the Middle East, Asia and parts of Africa.

In this study, the methanol extract of *L. inermis* exhibited a higher degree of antimicrobial activity as compared with ethanol

Table 1. Activity of *Lawsonia inermis* leaf extracts and controls against pathogenic microorganisms

No	Organism	Diameter of zone of inhibition (mm) (Including 6mm of disc size)				
		Hexane	Ethanol	Methanol	Gentamycin	Nystatin
1	<i>S. aureus</i>	0	18	30	15	-
2	<i>S. epidermidis</i>	0	0	0	10	-
3	<i>K.pneumonia</i>	0	13	19	20	-
4	<i>E. faecalis</i>	0	0	20	0	-
5	<i>E. cloacae</i>	0	0	23	17	-
6	<i>E. coli</i>	0	0	16	22	-
7	<i>P. aeruginosa</i>	0	0	16	10	-
8	<i>C. albicans</i>	0	0	26	-	18±0

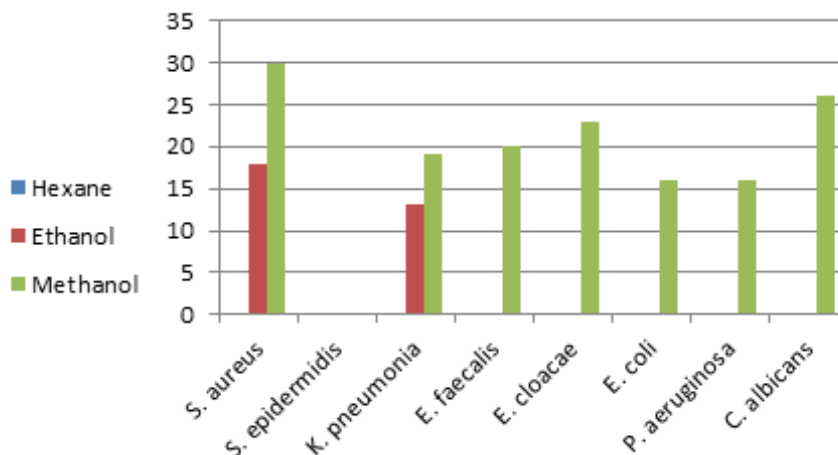


Fig. 1. Activity of *Lawsonia inermis* leaf extracts against pathogenic microorganisms

Table 2. Activity of *Lawsonia inermis* leaf extracts against pathogenic microorganisms MIC (mg/ml)

<i>S. aureus</i>	<i>S. epidermidis</i>	<i>K.pneumonia</i>	<i>E. faecalis</i>	<i>E. cloacae</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
1.5	300	6.25	6.25	6.25	12.5	12.5	3.12

and hexane extracts. Methanol as a solvent is the most commonly used for preliminary studies of antimicrobial activities in plants²¹. This higher antibacterial and antifungal activities of methanol extract is due to the nature of biological active components (alkaloids, flavonoids, terpenoids, tannins etc.), which may be more soluble in methanol. The stronger extraction capacity of methanol that may have yielded a greater number of active constituents responsible for the activities²². Several studies have been found that methanol is a best solvent for extraction of the active compounds^{23,24} as compared with ethanol, hexane and chloroform. The strains of *S. aureus* and *C. albicans* were the most susceptible to plant extracts. On the contrary, *E. coli* and *P. aeruginosa* were the most resistant microorganisms. This result is in accord with former studies that confirmed that gram positive bacteria are more sensitive to antimicrobial compounds than gram negative bacteria. The methanol extract of *Lawsonia inermis* showed broad activity against gram positive and gram negative bacteria, and yeast except *S. epidermidis*. The microorganism *E. coli*, which is already known to be multi-resistant to drugs, was inhibited by the methanol extracts of henna.

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On the other hand, *Pseudomonas aeruginosa* and other *Pseudomonas* sp, have always been important causes of hospital infection because of their intrinsic resistance to most antibiotics and ability to survive and even multiply at low temperatures and in disinfectant solutions, were inhibited by the methanol extracts. Such results are very interesting, because *E. coli* and *P. aeruginosa* are important nosocomial pathogens, resistant to antibiotics and its control is very difficult by therapeutic means. The antifungal activity of *L. inermis* leaf extract was evaluated *in vitro* against fungi such as *C. albicans*, *S. cerevisiae*, and dermatophytes by several investigators^{21, 25}. A study of Muhammad and Muhammad (2005)¹⁴ demonstrated the activity of *L. inermis* leaves against the hospital strains *A. niger* and *F. oxysporum* in addition to *Streptococcus* sp. and *S. aureus*. The aqueous and chloroform extracts of henna leaves were effective in inhibiting the growth of microorganisms that are involved in causing burn wound infections. On the other hand, some studies suggested that henna plant has a wide spectrum of antimicrobial activity including antibacterial, antiviral, antifungal and antiparasitic activities. Kannahi and Vinotha (2013)²³ found that the ethanol extract was shown significant

activity against yeasts (*Candida albicans* and *Saccharomyces cerevisiae*) compared to petroleum ether extract. In study of Suleiman and Mohamed (2014)²⁶ the antifungal activity of *L. inermis* leaf extract was evaluated *in vitro* against *C. albicans*, *S. cerevisiae*, and some selected dermatophytes. Petroleum ether extract showed a wide antifungal spectrum compared to ethanol extract against tested yeasts. Many reports cite the inhibitory activity of henna plant against gram negative and gram positive organisms. The extracts of tested plants showed a great activity in inhibiting the growth of bacteria and fungi, probably due to the presence of active ingredients that inhibit bacterial and fungal growth. Henna plant contains Lawsone in about 0.5 to 1.5% of its ingredients. Lawsone (2-hydroxynaphthoquinone) is the main constituent responsible for the dyeing properties of the plant. However, henna also contains mucilage, flavonoids, naphthalene derivatives, coumarins, tannic acid, luteolin and gallic acid¹². Antimicrobial activity may be due to more than one component. Investigations showed that henna plant is effective against different microorganisms especially against *P. aeruginosa*. In nosocomial infection, *Staphylococcus aureus* is one of the most prevalent microorganisms worldwide. Methicillin resistant strains represent 15-45% of all *Staphylococcus aureus* isolates²⁷. Inhibitory activity of henna was shown against both gram negative and gram positive microbes. In one study²⁸ Lawsone, displayed inhibitory effects against common nosocomial urinary tract pathogens such as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at certain concentrations. Recent data indicate that *E. coli* is the most common etiologic gram-negative organism, followed by *P. aeruginosa*, *Klebsiella species*, and *enterobacter species*²⁹.

In fact, Gram-negative bacteria are frequently reported to have developed multi-drug resistance to many of the commercially available antibiotics of which *E. coli* and *P. aeruginosa* are the most prominent. However, methanol extract of *L. inermis* is of special interest for further investigation as it showed activity against both gram-negative bacteria *E. coli* and *P. aeruginosa*.

CONCLUSIONS

According to the present study, *L. inermis* extracts showed varying degrees of antimicrobial activity against pathogenic microorganisms contributing to nosocomial infections. *L. inermis* plant was effective to combat the pathogenic microorganisms studied especially, *S. aureus* and *C. albicans*. Thus, the plant could be used as alternative source of antibacterial and antifungal agents. Further work is needed to isolate the secondary metabolites from the methanol extract of *L. inermis* in order to test specific activities and toxicity against animal and human cells.

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