



Original Research Article

Antimicrobial activity of *Lawsonia inermis* leaf extracts against some human pathogens

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ABSTRACT

Keywords

Antimicrobial activity; inhibition zone; solvent extracts; henna leaves

In this present study, henna leaves were collected and selected for antimicrobial activity against some human pathogens. Among this study, henna leaves were extracted with methanol, ethanol and aqueous by solvent extraction method. By this study, the pathogens are isolated from wound sample. The isolated organisms were identified based on cultural, morphological and biochemical characteristics. Hence, the isolated bacterial isolates were confirmed as *S.aureus*, *S.mutans*, *P.aeruginosa* and fungal isolates such as *A.niger*, *A.flavus* and *Fusarium*. In this study, antimicrobial activity was performed by disc diffusion and well diffusion method. The maximum activity was showed in methanol extraction against all isolated human pathogens. In this ethanol extraction also shows maximum activity (100%). There was no activity in aqueous extract. This result may open important perspectives alternative antimicrobial therapies.

Introduction

Medicinal plants are potential renewable natural resources and are generally considered to play a beneficial role in human health care. Since the dawn of civilization, medicinal plants are known as nature's best gift to cure a number of diseases of men and animals. There about 121 clinically useful prescription drugs that is worldwide derived from higher plants. About 70 percent of them came to the attention of pharmaceutical houses because of their use in traditional medicine (Abelson, 1990).

In the past, medicinal plants were used intensively in folkloric medicine for treatment of various disorders. Today, it is estimated that about 80 % of people in developing countries rely on traditional medicines for their primary health care. Traditional medicines are becoming popular, due to high toxicity and adverse effects of orthodox medicament. This has led to sudden increase in the number of herbal industries in the drug market. Several plant species are used by various indigenous systems such as Siddha,

Ayurveda, Unani and Allopathy for the treatment of different ailments. This review emphasizes the traditional uses and biological properties of *Lawsonia inermis* (Farnsworth *et al.*, 1985).

Lawsonia inermis (Lythraceae) is a perennial plant commonly called as henna, having different vernacular names in India viz., Mehndi in Hindi, Mendika, Rakigarbha in Sanskrit, Mailanchi in (Kirtikarkr and Basu, 1956). It Malayalam, Muruthani in Tamil, Benjati in Oriya, Mayilanchi in kannada and Mehedi in Bengali [is native to North Africa and South East Asia, and often cultivated as an ornamental plant throughout India, Persia, and along the African coast of the Mediterranean sea (Malekzadeh, 1968). Henna grows better in tropical savannah and tropical arid zones, in latitude between 15° and 25° N and S, produces highest dye content in between temperature 35 to 45°C. The optimal soil temperature ranges for germination are 25 to 30°C.

Henna leaves are very popular natural dye to color hand, finger, nails and hair. The dye molecule, Lawson is the chief constituents of the plant; its highest concentration is detected in the petioles (0.5-1.5%). In folk medicines, henna has been used as astringent, antihemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive, sedative and also as therapeutic against amoebiasis, headache, jaundice and leprosy (Raja sekaran, 2001).

India is one of the pioneers in the development and well documented indigenous systems at medicine, namely Ayurveda, Siddha and Unani. For millennia, the India population has depended upon mostly plant based crude

drugs for the treatment of variety of human ailments. India has about 18, 000 species of angiosperms, of which about 2,500 species are considered as important source of medicinal and aromatic chemical components (Cown, 2009).

In modern medicine also, plants occupy a very significant place as raw material for some important drugs although synthetic drugs and antibiotics brought about a revolution into controlling different disease. But these synthetic drugs are out of reach to millions of people. Traditional medicines are used by about 60 percent of the world's population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well as modern medicinal plants, minerals, and organic matter, the herbal drugs are prepared from medicinal plants only.

The main interest in plants because of their medicinally and pharmacologically important active ingredients rapidly. Medicinal plant products have been studied extensively and their role in several useful biological activities, such as antibacterial, antifungal, insecticides and herbicides have been well documented number of plant products with the useful bioactive properties is increasing rapidly as an outcome of several ongoing research programs on investigation of biological activities of a number of plants. These bioactive compounds have several as lead molecules for the development of many synthetic antibiotics. Hence, the present study was undertaken with the preparation of different solvents extracts of *Lawsonia inermis* and determination of antimicrobial activity of its leaf extracts by disc diffusion and well diffusion method.

Materials and Methods

Sample Collection

Wound sample (specimen) was collected with the help of specialist using sterile swabs. The swabs were immediately immersed into normal saline and transported to the laboratory. The specimen should be collected before any antimicrobial agents were administered.

Isolation and identification of pathogens

The swabs were immediately inoculated on Meconkey agar, Mannitol salt agar, Kings B medium which were incubated at 37^o C for 18 – 24 hrs.

Collection of plant material

The plant materials used for this study were collected from the rural area of Mahathevappatanam, Thiruvarur District, Tamil Nadu, South India.

Plant Extract preparation

The shade dried plant materials were pulverized into coarse powder and extracted in soxhelt apparatus using methanol, ethanol and aqueous extracts were also prepared. The collected methanol, ethanol and aqueous extracts were concentrated under vacuum (50^oC) dried and weighed for methanol, ethanol and aqueous extracts taken at different concentration 25%, 50%, 70% and 100% against test pathogens.

Antibacterial Activity

Disc Diffusion method (Perz *et al.*, 1990) Antibacterial activity of the extracts was tested by Disc-diffusion method (Kirby and Bauer, 1966). 0.2 ml bacterial cultures

(4hrs) were uniformly spread on solidified nutrient agar medium. The filter paper discs prepared with plant extracts were carefully placed over the spreaded cultures by using sterile needle and incubated at 37^o C for 24hrs. After the incubation period, the plates were examined for inhibitory zones (including the diameter of the disc) (Gupta *et al.*, 1993).

Well Diffusion Technique (Bauer *et al.*, 1966)

24 hrs old bacterial cultures were used for well diffusion method. One well of 5 mm size will be made in the help of sterile cork borer under aseptic condition in laminar air flow chamber. The wells were loaded with different concentration such as 25%, 50%, 75%, and 100% of the leaf extracts (5g in 100ml of solvent). The plates were incubated at 37^oC for 24 hours. The plates were observed after 24 hrs for clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well. The reading were taken in all the replicates the average values were tabulated (Baues *et al.*, 1996).

Antifungal Activity

Disc Diffusion method (Perz *et al.*, 1990)

Potato-Dextrose Agar medium was prepared and transferred to sterile petri dishes and allowed to solidify. A suspension of identified three fungal spp was added to media and swabs the entire surface of the agar medium separately. The inoculums were equally distributed in surface of the media by rotating the plate. Sterile 5mm disc in diameter dipped in solutions of the various solvent ethanol, methanol and aqueous plant extracts. The plates were left for 1 hour at room temperature as a period of pre incubation

diffusion to minimize the effects of variation in time between the applications of the different solutions. Then the plates were incubated at 24°C for 2-3 days and observed for antimicrobial activity. The diameters of the zone of inhibition was calculated and compared with the standards (Gupta, 1997).

Well Diffusion Technique (Bauer *et al.*, 1966)

The fungal culture used for well diffusion method. The Sabouraud agar plates were prepared and the fungal spores were spreaded on the Sabouraud agar plates. One well of 5mm, size made in the agar plates with the help of sterile cork borer under aseptic condition in laminar air flow chamber. The well were loaded with 0.2ml of leaf extracts. The plates were incubated at 28°C for 48hrs.

The plate was observed after 48 hrs for clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (Alexopolous and Mims, 1993).

Statistical Analysis

Random sampling was used for the entire test. The data of all the parameters were statistically analyzed and expressed as Mean \pm SD by using the formula given by (Essawi and Srour, 2000).

$$\text{Mean (X)} = \frac{\sum X}{N}$$

Where,

$\sum X$ = Sum of all values of variable

N = Number of observation

$$\text{Standard deviation} = \sqrt{\frac{\sum (x - \bar{x})^2}{N}}$$

Where,

$\sum (X - \bar{X})^2$ = The sum of the square of the deviation of each value from the mean.

N = Number of observation.

Results and Discussion

In the previous studies has been found to be a best solvent for extraction of the active ingredient (β asarone) from leaves. Though, solvents such as methanol, ethanol and aqueous used the most of the previous studies were also found to be suitable for extraction of active ingredients. It is well established that the β - asarones found in leaf, roots and rhizomes tissues are responsible for almost all of the antimicrobial activities of the *Lawsonia inermis* (MacGaw *et al.*, 2002).

The ethanol and water extract of the bark of *Lawsonia inermis* showed antimicrobial activity against gram positive and gram negative bacteria Diacyl heptenoid and its Fyrosdia crosyenin were identified at the constituent responsible for this activity (Saxena *et al.*, 1990). In this work, aqueous extracts of *Lawsonia inermis* inhibits the skin pathogens *S.aureus*, *S.mutans* and *P.aeruginosa* potentially at 100% concentration. this extracts control the bacteria and fungal pathogen which cause skin disease.

The antibacterial activity may be attributed to not only a single active principle but to a cocktail of a variety of active principles or alkaloid (Britto, 2001; Eassawi and Srour, 2000; Bisignano *et al.*, 2000. Osawa *et al.*, 2000)

Several recent papers reported that the presences of antibacterial activity are due to flavonoides (Harborne, 1988). Alkaloids are important defence of the plants against pathogenic organisms and herbivores. It also toxin for insects which further modify the alkaloids and incorporate them into their own defense secretion.

The present study has been under taken to find out the effectiveness of the different extracts of *Lawsonia inermis* leaves against some human bacterial and fungal pathogens.

Isolation of organisms

Nutrient agar and PDA plates were prepared. Then the wound samples were inoculated in to the agar plates. The bacterial colonies were identified by gram's staining and biochemical test where as fungi by lactophenol cotton blue mounting technique.

Antimicrobial activity

The antimicrobial potency of *Lawsonia inermis* leaf extracts were tested against some human pathogenic bacteria and fungi was quantitatively assessed for the presence or absence of zone of inhibition. The results relative to antibacterial activity was observed by measuring the diameter of the zone of inhibition.

Antibacterial activity of *Lawsonia inermis* by Disc diffusion method

The activity of methanol extracts of *Lawsonia inermis* leaves against *Staphylococcus aureus* showed minimum activity (2.3 ± 2.01 mm) at 25 % concentration and maximum activity (9.3 ± 8.9 mm) at 100% level. The activity

of methanol extracts of *Lawsonia inermis* against *Streptococcus mutans* showed minimum activity (2.3 ± 1.516 mm) at 25% followed by 50% (3.6 ± 3.23 mm), 75% (5 ± 5.12 mm) and 100% (17 ± 16.04 mm). The activity of methanol extracts of *Lawsonia inermis* against *pseudomonas aeruginosa*, showed maximum activity was obtained at 75% (4.6 ± 3.16 mm) followed by 100% (3.3 ± 2.16 mm), 50% (2.8 ± 2.4 mm) and 25% (2.6 ± 2.1 mm). The ethanol extracts of *Lawsonia inermis* leaves against *Staphylococcus aureus* showed minimum activity (3.1 ± 3.21 mm) at 25% concentration and maximum activity (8.1 ± 6.2 mm) at 100% level.

The ethanol extracts of *Lawsonia inermis* leaves against *Streptococcus mutans* showed minimum activity (2.0 ± 4.31 mm) at 25 % concentration maximum activity (9.1 ± 5.1 mm) at 100%. The ethanol extracts of *Lawsonia inermis* leaves against *pseudomonas aeruginosa* showed minimum activity (9.1 ± 8.61 mm) at 25% concentration and maximum activity (7.6 ± 6.41 mm) at 100%. There was no activity showed by aqueous extracts of *Lawsonia inermis* against *Staphylococcus aureus*, *Streptococcus mutans*, and *Pseudomonas aeruginosa*. The present investigation has been undertaken to find out the effectiveness of the methanol, ethanol and aqueous extracts of *Lawsonia inermis* against some human pathogenic microorganism such as *S.aureus*, *S.mutans*, *P.aeruginosa*, *A.niger*, *A.flavus* and *Fusarium*.

Antifungal activity of *Lawsonia inermis* By Disc diffusion method

The methanol extracts of *lawsonia inermis* leaves showed maximum activity at 100% level (2.5 ± 2.3 mm) and minimum activity

Table.1 Antibacterial activity of methanolic extracts of *Lawsonia inermis* (mm)

S.No	Organisms	Zone of inhibition(mm)			
		25%	50%	75%	100%
1	<i>S.aureus</i>	2.3±2.01	2.3±3.0	2.8±2.7	2.5±2.3
2	<i>S.mutans</i>	2.3±2.0	2.4±3.1	5.1±4.0	6.6±4.4
3	<i>P.aeruginosa</i>	2.6±2.1	2.8±2.4	4.6±3.16	3.3±2.16

Value are triplicate and represented as mean ± standard deviation

Table.2 Antibacterial activity of ethanolic extracts of *Lawsonia inermis* (mm)

S.No	Organisms	Zone of inhibition(mm)			
		25%	50%	75%	100%
1	<i>S.aureus</i>	3.1±3.21	2.3±3.0	2.8±2.7	8.1±6.2
2	<i>S.mutans</i>	2.0±4.31	2.4±3.1	5.1±4.0	9.1±4.1
3	<i>P.aeruginosa</i>	2.6±2.1	2.8±2.4	7.6±6.4	9.1±8.61

Table.3 Antifungal activities of methanolic extracts of *Lawsonia inermis* (mm)

S.No	Organisms	Zone of inhibition(mm)			
		25%	50%	75%	100%
1	<i>A.niger</i>	0.3±1.1	2.3±3.0	2.8±2.7	2.5±2.3
2	<i>A.flavus</i>	8.3±9.1	4.4±3.1	5.1±4.0	2.5±8.1
3	<i>Fusarium</i>	2.6±2.1	2.8±2.4	4.6±3.16	4.7±4.4

Value are triplicate and represented as mean ± standard deviation

Table.4 Antifungal activity of ethanolic extracts of *Lawsonia inermis* (mm)

S.No	Organisms	Zone of inhibition(mm)			
		25%	50%	75%	100%
1	<i>A.niger</i>	3.5±1.4	2.3±3.0	2.8±2.7	2.03±3.03
2	<i>A.flavus</i>	8.3±9.1	3.04±5.5	5.1±4.0	7.4±6.5
3	<i>Fusarium</i>	2.6±2.1	6.1±9.5	4.6±3.16	9.1±8.9

Value are triplicate and represented as mean ± standard deviation

at 25 % (0.3±1.1mm) against *A.flavus*. The methanol extracts of *Lawsonia inermis* leaves showed maximum activity at 100% (2.5.0±8.1mm) minimum activity at 25% (8.3±9.1mm) against *A.niger*. For *Fusarium* also it showed maximum activity of methanol extracts at 100% (4.7±4.4mm), followed by 25% (1.3±1.8mm), 50% (2.2±2.1mm) and 75% (1.3±1.8mm). The ethanol extracts of *Lawsonia inermis* showed maximum

activity at 100% (3.5±1.4mm) and minimum activity at 25% (2.03±3.03mm) against *A.flavus*. Ethanol extracts of *Lawsonia inermis* showed maximum activity at 100% (7.4±6.5mm) and minimum activity at 50% (3.04±5.5mm) against *A.niger*. The ethanol extracts of *inermis* showed maximum activity at 100% (9.1±8.9mm) and minimum activity 50% (6.1±9.5mm) against *Fusarium*. There was no activity showed by aqueous

extracts of *Lawsonia inermis* against *Staphylococcus aureus*, *Streptococcus mutans*, and *Pseudomonas aeruginosa*.

Well Diffusion method

The same results were obtained by well diffusion method for different against all organisms. Overall results of this study revealed that maximum activity of the leaf extracts of *Lawsonia inermis* was observed at 100% level against both bacteria and fungi. So usage of the extracts at a concentration of 100% might be effective to treat the some human pathogens (Table.4) *Aspergillus* is pathogenic to humans. It causes *Aspergillosis*. Out of eight species of *Aspergillus*, *Aspergillus niger*, *fumigates* account for most infections.

The antimicrobial activity potency of *Lawsonia inermis* leaves extracts were tested against human pathogenic microorganism and the quantitatively assessed for the presence or absence of zone of inhibition.

The humble *Lawsonia inermis* has value in the treatments of acne. The seeds are used, which contain proteins, lecithins, carbohydrates and fats. It is nutritious and anti dermatitis agent. They have been used in cases of acne and on faded, wrinkled skins [14]. In the present study *Lawsonia inermis* leaf extract was studied for their antiseptic and vasodilator activities and antimicrobial activity against skin disease causing pathogens.

In this present study, henna leaves were collected and selected for antimicrobial activity against some human pathogens. Among this study, henna leaves were extracted with methanol, ethanol and aqueous by solvent extraction method. By

this study, the pathogens are isolated from soil. The isolated organisms were identified based on cultural, morphological and biochemical characteristics. Hence the isolated bacterial colonies were confirmed as, *S.aureus*, *S.mutans*, *P.aeruginosa* and fungal colonies such as *A.niger*, *A.flaves* and *Fusarium*. By this study, antimicrobial activity was performed by disc diffusion and well diffusion method. The maximum activity was showed in methanol extraction against all isolated human pathogens. In this ethanol extraction, also shows maximum activity (100%). There was no activity in aqueous extract. This result may open important perspectives alternative antimicrobial therapies. In this studies are required to study the effect and toxicity of these compounds in experimental animals (in vivo) and to establish if they could be safety used as antibacterial agent against the pathogens. Thus we concluded that the medicinal plants could be used as drug to treat the infections caused by bacteria and fungi without any side effects.

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