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Research Article

IN VITRO ANTIBACTERIAL ACTIVITIES OF *LAWSONIA INERMIS* LEAF EXTRACTS

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ABSTRACT
To know the *in vitro* antibacterial activities of n-hexane, chloroform, ethyl acetate, benzene, acetone and acetronitrile extracts of the leaves of *Lawsonia inermis*(Family- Lythraceae), present study was conducted. All the extracts were used at 50mg/ml, 100mg/ml and 300mg/ml concentrations. For the present study *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* were used. Against *Bacillus subtilis*, the ethyl acetate extract was more potent than tetracycline (25µl/ml), which was used as a positive control. On the other hand, the chloroform extract and tetracycline more or less produce equal zone of inhibition against that strain. n-hexane, chloroform, ethyl acetate, acetone and acetonitrile extracts were effective against all the strains used in the study. All the extracts of *Lawsonia inermis*, which were having antibacterial activities, produced such activities in a dose dependent manner. For the study dimethylsulfoxide was used as negative control.
Keywords: Antibacterial activity, *Lawsonia inermis*, Extracts, Tetracycline, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*

INTRODUCTION

Natural drugs are obtained from the plant, animal or mineral kingdom. The plant kingdom is the store house of the organic compounds[1]. *Lawsonia inermis* Linn (Family-Lythraceae) is commonly known as henna and mehendi. It is a small shrub to a height of 6m, the branches of this plant are lateral with leaves that grow in pair which are 2-4cm long. The flowers are fragrant and red rose like[2]. Henna leaves contain an important pigment called "lawsone". It also contains mannite, tannic acid, gallic acid, mucilage and napthaquinone. It is also known as good medicinal plant which is said to have properties of astringent, antihaemorrhagic, intestinal, neoplastic, hypotensive and sedative effects[3]. Useful parts of the plant are leaf, flower, bark, root and seed. The leaf used for alleviating jaundice, skin disease, veneral disease and small pox. Seeds are effective against dysentery, liver disorders and associated problems. Bark is used for burn scald. Root is considered as a potent medicine for gonorrhoea and herpes[4].

Some workers have mentioned that ethanolic, petroleum ether, ethyl acetate, methanolic and water extracts of leaves of *Lawsonia inermis* are affective *in vitro* against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli, Proteus vulgaris,* and *Klebsiella pneumonia*[5]. Moreover, some other researchers also indicated that ethanol, petroleum ether and chloroform extracts of the leaves of the plant show antimicrobial activity only against *Staphylococcus aureus, Bacillus subtilis* and *Escherchia coli*. They have also followed disc diffusion method[6].

In addition, some other researchers also indicated that n-hexane, chloroform and methanol extracts of the leaves of the plant displayed antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. They followed agar well diffusion method[7]. Moreover, the antibacterial activity of aqueous methanolic and water extracts of the leaves of the plant showed antibacterial activity against *S.aureus, B.cereus* and *E.coli*[8].

Considering it as a potential antibacterial agent, we under took antibacterial activity study using n-hexane, chloroform, ethyl acetate, benzene, acetone and acetonitrile extracts of leaves of *Lawsonia inermis.*

MATERIALS AND METHODS

Plant Material:-

The leaves of the plant *Lawsonia inermis* were collected from Chhend, Rourkela, during December 2011. The sample was authenticated by Dr. Prativa Sahoo, Botanist, Rourkela Autonomous College, Rourkela. The shade dried leaves were powdered and stored in a dessicator until evaporation.

Preparation of Extracts:-

The powdered leaves were passed through a sieve (No.40) and stored in a dessicator. The powdered leaves (10gm) of *Lawsonia inermis* were extracted by using maceration method. The powdered leaves were macerated in 60ml of n-hexane for 3 days at room temperature. The resulting extract was filtered through a filter paper (Whatman No.1). The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness under reduced pressure. Instead of using n-hexane, the above mentioned procedure was conducted separately for chloroform, ethyl acetate, benzene, acetone and acetonitrile[9].

Agar well Diffusion Method:-

In order to determine the *in vitro* antibacterial activity of the dry extracts (dissolved in DMSO) of n-hexane, chloroform, ethyl acetate, benzene, acetone and acetonitrile extracts of *Lawsonia inermis* leaves, the nutrient agar well diffusion method as described by Schillenger and Luke(1989) was performed. Sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight nutrient broth culture of *Staphyloccus aureus* (approx. 10^7CFU/ml) and poured into sterile petriplates. In each plate, five wells of 6mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5min. In one well, 50µl of n-hexane extract (50mg/ml) was poured. In other wells, 50µl each of 100mg/ml n-hexane extract, 300mg/ml n-hexane extract, tetracycline (25µl/ml) as a positive control[10] and DMSO (as a negative control) were delivered. Same procedure was followed for other extracts. They were used at 50mg/ml, 100mg/ml and 300mg/ml concentrations[7]. Similar procedure was performed using other two strains such as *Bacillus subtilis* and *Escherichia coli*. After holding the plates at room temperature for 2 hours to allow diffusion of the extracts and controls into the nutrient agar medium, they were incubated

at 37^0c for 24 hours. They were examined for inhibition of the bacterial growth. The diameters of the zone of inhibition in each case were measured[11].

RESULTS

While chloroform extract showed minimum efficacy against *Staphylococcus aureus*, acetone extract displayed maximum antibacterial activity against it. All the extracts were less potent than the positive control in case of S*taphylococcus aureus. (*Table-1)

Against *Bacillus subtilis*, benzene extract did not show any antibacterial activity, while n-hexane, acetone and acetonitrile extracts were more or less equally effective against it. Ethyl acetate extract was more potent than positive control whereas chloroform extract and tetracycline were more or less equally effective. (Table-2)

All the extracts were less potent than the positive control against *Escherichia coli,* while benzene extract was totally inactive. (Table-3)

The negative control (DMSO) of the study did not show any antibacterial activity. All the extracts of *Lawsonia inermis*, which were having antibacterial activities, produced such activity in a dose-dependent manner.

DISCUSSION

Till now few researchers have worked on antibacterial activity study taking leaf extracts of *Lawsonia inermis*. Like El-Kamali et al. (2009), Akter et al. (2010), Mastanaiah et al.(2011) and Hussain et al.(2011), we have found that ethyl acetate, chloroform and n-hexane extracts were effective against all the strains used in the study[5-8]. Moreover, we have seen that ethyl acetate extract was more potent than the positive control against *Bacillus subtilis*. Some other extracts also showed antibacterial activity against it. In addition, chloroform extract was more or less as potent as positive control against *Bacillus subtilis*. While benzene extract was ineffective.

CONCLUSION

The extracts used in this investigation have displayed variable antibacterial activities most probably it is due to the differences in the phytochemical constituents extracted by the different solvents used in our study. Systematic screening of plant using several extracts from the different parts of the plant (instead of taking pure compounds) may lead to the discovery of novel active compounds.

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Table-1 Antimicrobial activity pattern of extracts and controls against *Staphylococcus aureus*

Extracts	Zone of inhibition after 24 hours (in mm)				
	Concentration (mg/ml)			Tetracycline 25(µl/ml)	DMSO
	50	100	300		
n-hexane	15	17	20	32.4	0
Chloroform	14	16	18	32.4	0
Ethyl acetate	17	18	23	32.4	0
Benzene	15	17	19	32.4	0
Acetone	22	24	30	32.4	0
Acetonitrile	19	23	25	32.4	0

Table-2 Antimicrobial activity pattern of extracts and controls against *Bacillus subtilis*

Extracts	Zone of inhibition after 24 hours (in mm)				
	Concentration (mg/ml)			Tetracycline 25(µl/ml)	DMSO
	50	100	300		
n-hexane	15	22	24	25.25	0
Chloroform	20	23	25	25.25	0
Ethyl acetate	30	32	37	25.25	0
Benzene	0	0	0	25.25	0
Acetone	18	20	22	25.25	0
Acetonitrile	16	19	21	25.25	0

Table-3 Antimicrobial activity pattern of extracts and controls against *Escherichia coli*

Extracts	Zone of inhibition after 24 hours (in mm)				
	Concentration (mg/ml)			Tetracycline 25(µl/ml)	DMSO
	50	100	300		
n-hexane	12	14	16	24.5	0
Chloroform	0	0	12	24.5	0
Ethyl acetate	0	13	16	24.5	0
Benzene	0	0	0	24.5	0
Acetone	14.5	18	19	24.5	0
Acetonitrile	15	18	20	24.5	0

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