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RESEARCH ARTICLE

Antibacterial Potential of Selected Medicinal Plants against the Human Pathogens

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Abstract

To screen the antibacterial potential of selected medicinal plants. The antibacterial activity of selected medicinal plants were investigated by disc diffusion method against eight species of bacteria *viz., Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Streptococcus pyogens* and *Bacillus subtilis.* Methanol extract of *Strychnos nux-vomica* showed the higher antibacterial activity against all the tested organisms when compared to *Lantana camara*. The methanol extract of *Strychnos nux-vomica* was considered for the further spectrum analysis.

INTRODUCTION

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to search for biologically active principles in plant. Medicinal plants represent a rich source of antimicrobial agents. Wide range of different parts of medicinal plants was used for extracts as raw drugs and they possess the varied medicinal properties **(Renisheya Joy Jeba Malar *et al*., 2011)**. Some of these raw drugs were collected in smaller quantities for local use while many other raw drugs are collected in the larger quantities and may traded in market as raw materials to many herbal industries. Our nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants growing wild in different parts of our country. In India several medicinal plants are used since time immemorial to cure specific ailments **(Utpal Chandra De *et al*., 2014)**.

Usually the discovery of new drugs to cure pathogenic infections is done by using pathogens which was obtained from the commercial laboratories. The screening of drugs by using such microbial strains was comparatively for many years which were not effective due to the evolution of resistant strains at high rate. So, the antibacterial screening of any drug by using freshly collected clinical sample will be more potent **(Vadnere Gautam *et al*., 2013)**. The revival of interest in plant derived drug is mainly due to the current wide spread belief that "green medicine" was safe and more dependable than the costly synthetic drugs which may have adverse effect. Hence, researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against microbial infections.

Materials and Methods

Collection of plants

Healthy and well grown leaves of selected plants *viz., Lantana camara* and *Strychnos nux-vomica* were collected from Cuddalore district, Tamil Nadu, India. The leaves were immediately brought to the laboratory using separate sterile polythene bags. First they were washed with tap water, then surface sterilized in 10 per cent sodium hypochlorite solution to prevent the contamination of microbes **(Krishnan kannathasan *et al*., 2011)**, then rinsed with sterile distilled water and air dried in shade at room temperature. Voucher specimen (286 and 287) deposited as herbarium and authenticated in Department of Botany, Annamalai University, Tamilnadu, India.

Preparation of plant extracts

Forty grams of the powdered leaves were loaded in soxhlet apparatus and extracted in 125 mL of different solvents *viz.*, Hexane, chloroform, ethyl acetate, acetone and methanol. The extractions were evaporated at rotary evaporator at 40°C **(Vogel, 1978).**

Antibacterial assays

Disc diffusion method

The disc diffusion method **(Bauer *et al.*, 1966)** was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 mL of Mueller Hinton Agar and allowed to solidify for the use in susceptibility test against bacteria respectively. Plates were dried and 0.1 mL of standardized inoculums was poured and uniformly spreaded. The excess inoculums were drained and the plates were allowed to dry for 5 minutes. After drying, the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. Gentamycin (10 µg/disc) were used as the positive control and 5 percent DMSO was used as blind control in these assays. Finally, the inoculated plates were incubated at 37°C for 24 h. The zone of inhibition was observed and measured in millimeters.

Microorganisms used

The antibacterial activity of selected medicinal plants were investigated against eight species of bacteria *viz.*, *Escherichia coli* (MTCC 1696), *Salmonella typhi* (MTCC 3220), *Staphylococcus aureus* (MTCC 7443), *Pseudomonas aeruginosa* (MTCC 7454), *Klebsiella pneumonia* (MTCC 9751), *Proteus vulgaris* (MTCC 1771), *Streptococcus pyogens* (MTCC 1925) and *Bacillus subtilis* (MTCC 2390) were obtained from microbial type culture collection, Chandigarh, India. The stock cultures were maintained on Nutrient Agar medium at 4°C.

In vitro antibacterial activities were determined by using Mueller Hinton Agar and Mueller Hinton Broth and they were obtained from Himedia Ltd., Mumbai, India.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the plant extracts were tested in Mueller Hinton Broth by broth macro dilution method **(Ericsson and Sherris, 1971).** The plant extracts/fractions were dissolved in 5 per cent DMSO to obtain 500µg/mL. From the stock solution 0.5 mL of stock solution was incorporated into 0.5 mL of Mueller Hinton Broth to get a concentration of 500, 250, 125, 62.5, 31.25, 15.62, 7.6, 3.3 and 1.15 µg/mL for plant extracts and 50 µl of standardized suspension of the test organism was transferred onto each tube. The control tube contained only organism and devoid of plant extracts. The culture tubes were incubated at 37°C for 24 h. The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation was determined as MIC.

Minimum Bactericidal Concentration (MBC)

The MBC of the extracts were determined **(Kartnig *et al.*, 1991)** by plating 100 µL samples from each MIC assay tube with growth inhibition into freshly prepared Mueller Hinton Agar plates were incubated at 37°C for 24 h. The MBC were recorded as the lowest concentration of the extracts/fractions that did not permit any visible bacterial colony growth on the appropriate agar plate during the period of incubation.

Results

The antibacterial activity of methanol extract of *Lantana camara* in different concentrations against bacteria (*Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus pyogens and Bacillus subtilis*) are presented in Table 1. The mean zone of inhibition ranged between 6.5 and 12.2mm. For Gentamycin, the positive control, the zone of inhibition ranged from 18.0 to 20.0mm respectively. The MIC values of methanol extract ranged from 125 to 250 mg/mL, whereas the MBC values ranged from 250 to 500mg/mL. The highest mean zone of inhibition (12.2mm) was recorded with *Bacillus subtilis* against the methanol extract. For MIC *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Bacillus subtilis* showed lowest value (125mg/mL) and for MBC lowest value was (250mg/mL).

The antibacterial activity of methanol extract of *Strychnos nux-vomica* in different concentrations against bacteria (*Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus pyogens and Bacillus subtilis*) are presented in Table 2. The mean zone of inhibition ranged between 10.2 and 17.2mm. For Gentamycin, the positive control, the zone of inhibition ranged from 18.0 to 20.0mm respectively. The MIC values of methanol extract ranged from 62.5 to 250 mg/mL, whereas the MBC values ranged from 125 to 250mg/mL. The highest mean zone of inhibition (17.2mm) was recorded with *Staphylococcus aureus* against the methanol extract. For MIC *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Bacillus subtilis* showed lowest value (62.5mg/mL) and for MBC lowest value was (125mg/mL).

Table: 1 Antibacterial activity of Methanol extract of *Lantana camara*

S. No	Bacterial pathogens	Zone of inhibition (mm)			Gentamycin (10 µg/disc)	MIC µg/mL	MBC µg/mL
		100 µg/mL	200 µg/mL	300 µg/mL			
1.	*Escherichia coli*	7.7 ± 0.3	8.8 ± 0.3	11.5 ± 0.5	20	125	250
2.	*Salmonella typhi*	7.3 ± 0.7	8.5 ± 0.4	10.0 ± 0.4	19	125	250
3.	*Staphylococcus aureus*	7.0 ± 0.2	8.6 ± 0.4	10.7 ± 0.5	20	125	250
4.	*Klebsiella pneumoniae*	6.8 ± 0.6	8.2 ± 0.5	11.1 ± 0.4	18	125	250
5.	*Pseudomonas aeruginosa*	8.0 ± 0.4	9.5 ± 0.4	11.9 ± 0.2	20	125	250
6.	*Proteus vulgaris*	6.5 ± 0.2	8.9 ± 0.6	10.3 ± 0.5	19	250	500
7.	*Streptococcus pyogens*	6.8 ± 0.6	8.0 ± 0.2	10.8 ± 0.3	19	250	500
8.	*Bacillus subtilis*	8.6 ± 0.5	10.9 ± 0.3	12.2 ± 0.6	20	125	250

SD±value, mm- Zone of inhibition in millimeter, MIC- Minimum Inhibitory Concentration, MBC- Minimum Bactericidal Concentration

Table: 2 Antibacterial activity of Methanol extract of *Strychnos nux-vomica*

S. No	Bacterial pathogens	Zone of inhibition (mm)			Gentamycin (10 µg/disc)	MIC µg/mL	MBC µg/mL
		100 µg/mL	200 µg/mL	300 µg/mL			
1.	*Escherichia coli*	11.0 ± 0.3	13.4 ± 0.3	15.3 ± 0.5	20	62.5	125
2.	*Salmonella typhi*	10.2 ± 0.5	13.5 ± 0.4	14.0 ± 0.4	19	125	250

3.	*Staphylococcus aureus*	12.3 ± 0.2	14.6 ± 0.4	17.2 ± 0.5	20	62.5	125
4.	*Klebsiella pneumoniae*	10.4 ± 0.6	12.2 ± 0.5	14.7 ± 0.4	18	62.5	125
5.	*Pseudomonas aeruginosa*	11.3 ± 0.4	13.5 ± 0.4	15.5 ± 0.2	20	62.5	125
6.	*Proteus vulgaris*	12.5 ± 0.2	14.3 ± 0.6	16.3 ± 0.5	19	125	250
7.	*Streptococcus pyogens*	11.2 ± 0.6	13.0 ± 0.2	15.4 ± 0.3	19	125	250
8.	*Bacillus subtilis*	11.6 ± 0.5	13.9 ± 0.3	16.2 ± 0.6	20	62.5	125

SD±value, mm- Zone of inhibition in millimeter, MIC- Minimum Inhibitory Concentration, MBC- Minimum Bactericidal Concentration

Discussion

In the present investigation, among the plant extracts tested, the methanol extract of *Strychnos nux-vomica* was found to be more active against all the bacterial strains tested. The methanol extracts of *Strychnos nux-vomica* showed a broad spectrum of antibacterial activity against selected pathogens *viz.*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus pyogens* and *Bacillus subtilis*. In addition, the results of the present study confirmed the reports of previous studies that methanol is a better solvent for more consistent extraction of antibacterial substances from medicinal plants when compared to other solvents such as hexane, chloroform, ethyl acetate and acetone **(Lin *et al.*, 1999)**.

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