Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(4):1056-1059

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antimicrobial activity of Neem, Tulsi, Henna and Amla against pathogenic bacteria

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ABSTRACT

The present study investigatedthe antimicrobial potential of four medicinal plants viz., tulsi, amla, neem and henna against the pathogenic bacteria. The ethanolic extract of all medicinal plants exhibited maximum antimicrobial activity against all bacterial pathogens. The ethanolic extract of henna exhibited maximum antimicrobial potential against E. coli (19.6±0.27 mm) while that of amla exhibited maximum antimicrobial potential against E. coli (16.7±0.34 mm). The ethanolic extract of tulsi exhibited maximum antimicrobial potential against Klebsiella(13.3±0.47 mm) while the ethanolic extract of neem exhibited maximum antimicrobial potential against E. coli (15.4±0.20 mm).

Key words: Bacterial pathogens, Neem, Tulsi, Amla, Henna, Antimicrobial potential

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INTRODUCTION

The increasing resistance amongst the pathogenic bacteria against the antibiotics in recent years has pose serious problems in treatment [1, 2]. This has led a wide search to alternatives and in this race the medicinal plants have drawn a wide attention. The biologically active compounds present in the extract of medicinal plants show a significant antimicrobial potential [3, 4, 5]. Thus the medicinal plants are the new targets which can be exploited for development of new antimicrobial agents [4, 5]. The antimicrobial compounds present in the plant extracts need to be isolated, purified and identified. The identification of chemical structure of the active component present in these medicinal plants would help in designing a new chemical compound mimicking the natural compound but with better efficacy. This will have a great significance in treatment of infectious diseases. The present study was aimed at studying the antimicrobial activity of amla (*Emblica officinalis)*, neem (*Azadirachta indica)*, tulsi (*Ocimum sanctum)* and henna (*Lawsonia inermis)* plant extracts against pathogenic bacteria.

EXPERIMENTAL SECTION

2.1Bacterial culture

The pathogenic bacteria viz., *E. coli*, *Pseudomonas*, *Serratia*, *Alcaligenes* and *Klebsiella* were taken from culture collection center, department of microbiology, Dolphin (PG) Institute of Biomedical Sciences, Dehradun, India.

2.2 Preparation of plant extract

Leaves of neem (*Azadirachta indica)*, tulsi(*Ocimum sanctum)* and henna (*Lawsonia inermis)* plants and fruit of amla (*Emblica officinalis)* were collected and left to dry at room temperature for 24 hours. They were then grinded to a

fine powder and were kept in dry containers. The ethanolic extract was prepared by soaking each powder in 100% ethanol in a concentration of 1:4 for 24 hours. This mixture was cooled and filtered by Whatman filter paper No.1. The solvent was dried and concentrated using orbital shaker at 40°C. Water-based plant extracts were prepared in the same way except that distilled water was used instead of ethanol.

2.3 Evaluation of antimicrobial activity of extracts

The antimicrobial activity of extract against pathogenic bacteria was evaluated by using agar well diffusion method. The isolates were inoculated into 10mL of sterile Nutrient broth, and incubated at 37±1^{0}C overnight. The turbidity of culture was compared with Mac Farland standard number II. The cultures were swabbed on the surface of sterile Mueller-Hinton agar plates using a sterile cotton swab and allowed to dry for 3-5 minutes. Agar wells were prepared with the help sterilized borer with 10mm diameter. The extract of spices was diluted to give the final concentration 1000ppm, 2000ppm, 3000ppm and 4000ppm. 100 µl of different dilutions of the extracts was added to the wells of the inoculated plates. 50% ethanol and 50% methanol was used as control which was introduced into the well instead of the extract. The plates were incubated in an upright position at 37±1^{0}C for 24hrs. The zone of inhibition was measured and expressed in millimetres (mm).

RESULTS

All extracts of medicinal plants showed good antibacterial property (Table 3.1 to 3.4). The ethanolic extract of henna exhibited maximum antimicrobial potential against *E. coli* (19.6±0.27 mm) and least towards *Serratia* (10.6±0.67 mm) while the aqueous extract exhibited maximum activity against *E. coli* (16.7±0.34 mm) and least towards *Serratia* (10.7±0.25 mm).The ethanolic extract of amla exhibited maximum antimicrobial potential against *E. coli* (16.7±0.34 mm) and least towards *Serratia* (15.1±0.16 mm) while the aqueous extract exhibited maximum activity against *Alcaligenes*(14.2±0.18 mm) and least towards *Serratia* (9.2±0.15 mm).

Table 3.1a: Antimicrobial activity of ethanolic extract of henna against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	11.0±0.34	13.8±0.20	15.7±0.24	19.6±0.27
Pseudomonas	8.3±0.94	10.6±0.54	12.8±0.34	15.4±0.35
Klebsiella	10.7±0.26	12.6±0.22	14.7±0.45	16.6±0.35
Serratia	6.3±0.47	7.9±0.25	9.1±0.25	10.6±0.67
Alcaligenes	9.5±0.35	11.7±0.36	13.5±0.25	15.8±0.54

Values are mean ± SD of three replicates

Table 3.1b: Antimicrobial activity of aqueous extract of henna against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	**2000ppm**	**3000ppm**	**4000ppm**
E. coli	7.6±0.47	9.0±0.25	11.6±0.42	16.7±0.34
Pseudomonas	5.4±0.23	7.6±0.23	8.9±0.25	11.4±0.23
Klebsiella	7.6±0.34	9.7±0.35	11.8±0.24	13.6±0.28
Serratia	4.5±0.34	6.7±0.32	9.6±0.32	10.7±0.25
Alcaligenes	6.7±0.21	8.7±0.14	10.3±0.20	11.9±0.24

Values are mean ± SD of three replicates

Table 3.2a: Antimicrobial activity of ethanolic extract of amla against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	**2000ppm**	**3000ppm**	**4000ppm**
E. coli	9.3±0.34	11.7±0.32	14.3±0.42	16.7±0.34
Pseudomonas	7.0±0.82	8.7±0.47	9.4±0.56	10.0±0.81
Klebsiella	7.5±0.25	9.4±0.24	11.4±0.14	14.8±0.25
Serratia	5.7±0.28	7.3±0.15	9.5±0.17	12.9±0.23
Alcaligenes	11.7±0.24	12.6±0.35	13.6±0.24	15.1±0.16

Values are mean ± SD of three replicates

Table 3.2b: Antimicrobial activity of aqueous extract of amla against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	6.7±0.23	8.5±0.24	10.3±0.25	12.7±0.26
Pseudomonas	4.3±0.47	6.4±0.15	8.3±0.12	9.8±0.17
Klebsiella	5.7±0.47	8.3±0.42	10.3±0.14	12.6±0.22
Serratia	4.5±0.23	6.8±0.22	7.6±0.16	9.2±0.15
Alcaligenes	8.7±0.27	10.6±0.15	12.3±0.24	14.2±0.18

Values are mean ± SD of three replicates

Table 3.3a: Antimicrobial activity of ethanolic extract of tulsi against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	7.5±0.28	8.3±0.34	9.7±0.45	11.4±0.12
Pseudomonas	4.5±0.12	6.5±0.13	8.4±0.10	10.6±0.12
Klebsiella	7.3±0.12	9.3±0.47	11.3±0.25	13.3±0.47
Serratia	3.7±0.32	5.5±0.20	7.9±0.32	10.9±0.23
Alcaligenes	5.4±0.20	7.5±0.25	9.6±0.23	11.8±0.26

Values are mean ± SD of three replicates

Table 3.3b: Antimicrobial activity of extract aqueous of tulsi against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	5.7±0.14	7.3±0.32	8.7±0.20	10.4±0.14
Pseudomonas	3.5±0.14	4.7±0.12	6.7±0.25	8.3±0.15
Klebsiella	5.3±0.15	7.7±0.32	8.3±0.27	9.3±0.24
Serratia	2.7±0.24	4.5±0.28	6.3±0.23	8.7±0.47
Alcaligenes	4.7±0.21	6.7±0.23	8.6±0.27	10.7±0.34

Values are mean ± SD of three replicates

Table 3.4a: Antimicrobial activity of ethanolic extract of neem against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	2000 ppm	3000 ppm	4000 ppm
E. coli	8.3±0.23	10.6±0.21	12.7±0.23	15.4±0.20
Pseudomonas	7.3±0.24	9.2±0.23	11.4±0.34	13.6±0.47
Klebsiella	9.3±0.94	11.2±0.23	13.7±0.24	15.0±2.16
Serratia	6.4±0.26	8.5±0.47	10.3±0.18	12.6±0.40
Alcaligenes	8.5±0.27	10.4±0.15	12.4±0.22	14.4±0.20

Values are mean ± SD of three replicates

Table 3.4b: Antimicrobial activity of extract of aqueous neem against bacterial pathogens

Name of Organism	Zone of inhibition (mm)			
	1000 ppm	**2000 ppm**	**3000 ppm**	**4000 ppm**
E. coli	6.7±0.27	8.3±0.24	10.7±0.16	12.7±0.14
Pseudomonas	5.5±0.23	7.6±0.24	9.7±0.23	10.5±0.22
Klebsiella	6.5±0.21	8.7±0.23	10.7±0.15	12.4±0.24
Serratia	4.8±0.16	6.7±0.22	8.5±0.15	10.3±0.20
Alcaligenes	7.5±0.20	9.7±0.10	11.4±0.24	13.4±0.22

Values are mean ± SD of three replicates

The ethanolic extract of tulsi exhibited maximum antimicrobial potential against *Klebsiella*(13.3±0.47 mm) and least towards *Pseudomonas* (10.6±0.12 mm) while the aqueous extract exhibited maximum activity against *Alcaligenes*(10.7±0.34 mm) and least towards *Pseudomonas* (8.3±0.15 mm).The ethanolic extract of neem exhibited maximum antimicrobial potential against *E. coli* (15.4±0.20 mm) and least towards *Serratia*(12.6±0.40 mm) while the aqueous extract exhibited maximum activity against *Alcaligenes*(13.4±0.22 mm) and least towards *Serratia*(10.3±0.20 mm).

DISCUSSION

Infectious diseases are the cause of major deaths world-wide. The treatment is becoming difficult due to emergence of multi drug resistance amongst the pathogens[2, 6]. It is therefore imperative to search for natural compounds exhibit potent antimicrobial property. The medicinal plants are drawing increasing attention all over the world[7, 8, 9]. The active component present in these plant extracts can be formulated for drug preparation[10, 11, 12]. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation, damage protein, interfere with the enzymatic activities inside cell, affect synthesis of DNA and RNA, affect electron transport and nutrient uptake, leakage of cellular components, impair the energy production inside cell, change fatty acid and phospholipid constituents[11, 12].

The medicinal plants investigated in the present study showed good antimicrobial potential and were found to be most effective against gram-negative bacteria. The possible reason could be that they have less rigid and more porous cell wall as compared to that of gram-positive bacteria. The maximum antimicrobial potential was exhibited by ethanolic and aqueous extract of henna. The ethanolic extract of amla exhibited maximum antimicrobial potential against *E. coli* (16.7±0.34 mm) and least towards *Serratia* (15.1±0.16 mm) while the aqueous extract exhibited maximum activity against *Alcaligenes*(14.2±0.18 mm) and least towards *Serratia* (9.2±0.15 mm).The ethanolic extract of tulsi exhibited maximum antimicrobial potential against *Klebsiella*(13.3±0.47 mm) and least towards *Pseudomonas* (10.6±0.12 mm) while the aqueous extract exhibited maximum activity against *Alcaligenes*(10.7±0.34 mm) and least towards *Pseudomonas* (8.3±0.15 mm). The ethanolic extract of neem exhibited maximum antimicrobial potential against *E. coli* (15.4±0.20 mm) and least towards *Serratia*(12.6±0.40 mm) while the aqueous extract exhibited maximum activity against *Alcaligenes*(13.4±0.22 mm) and least towards *Serratia*(10.3±0.20 mm). Thus the ethanolic extract of these plants can be further investigated to identify the potent antimicrobial compound present which can serve as important candidate for drug formulation.

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