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Antibacterial Activity of Medicinal Plant *Crateava nurvala* (bark) against Bacterial Strains Causing Urinary Tract Infection

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Bacterial strains EC1, KL1 and PS1 used in the present study were isolated from patient's urine, suffering from urinary tract infection (U.T.I.) and were characterized as *E. coli*, *Klebsiella* sp. and *Pseudomonas* sp. respectively. Antibacterial activities in crude extract of bark *Crateava nurvala* (varuna) plant were tested against bacterial strains *in vitro*. The maximum growth inhibition by the crude extract was recorded against *E. coli* followed by *Pseudomonas* sp. and *Klebsiella* sp. after 48 h incubation at 28 ± 1°C being of 35.3 mm, 28.4 mm and 18.6 mm diameter respectively. As far as antibiotic sensitivity was concerned *E. coli* was maximum inhibited by amikacin, *Klebsiella* sp. and *Pseudomonas* sp. by ciprofloxacin, inhibition zone being 38.1 mm, 40.5 mm and 27.2 mm respectively after 24 h at 28 ± 1°C.

INTRODUCTION

Urine is a yellow, slightly acidic liquid consisting mainly of water, apart from urea, uric acid and nitrogenous waste products filtrated from blood by the kidney[1]. Urine is excellent culture medium for the multiplication of the urinary tract infection (UTI) causing bacteria. *E. coli, Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Proteus* sp. are the main causes of UTI.[2] UTI occurs more commonly in female than male due to anatomical and physiological differences.

C. nurvala (varuna) is a medicinal plant commonly distributed all over India and Burma. It is wild or cultivated, often found along streams but also in dry deep border formations in the sub-Himalayan tract. Plant bark is hot, bitter at first and then sweet sharp in taste, easy to digest and commonly used as medicine for treatment of many diseases of chest, blood, tuberculosis, fever, skin disease, and urinary tract infection. Plant bark also promotes appetite and decreases secretion of bile, phleum and removes disorder of the urinary complaints. It also relieves vomiting and other symptoms of gastric irritation. The fresh leaves are rubefacient and vesicant; they are remedies for swelling of feet and burning sensation and are equally useful in the antidotal treatment of snake bite[3]. Medicinal plants mainly in crude forms, arista's (liquor), avella, ghrit, tel, metals and minerals extensively processed with herbs are used for medicines for curing of several diseases. In the present study *C. nurvala* plant bark was checked for its efficiency against UTI causing bacteria with reference to synthetic antibiotics.

EXPERIMENTAL

Test sample: In total seventy-five urine samples (15 mL each) were collected in sterilized culture tubes at Sai Pathological Center, Haridwar to detect the presence of UTI causing bacteria. Three bacterial strains EC1, KL1 and PS1 were isolated from patients' urine samples by standard microbiological techniques on different culture media and were characterized according to Bergey's Manual of Determinative Bacteriology[4]. Strains were maintained on nutrient agar slant at 4°C temperature.

Plant bark of medicinal plant *Crateava nurvala* (varuna) was collected from Aurvedic Medical College and Hospital, Rishikul, Haridwar. Plant bark was kept in a dry place at 4°C temperature.

Extraction of crude extract: 150 g of bark powder was kept in a soxhlet extractor with sufficient quantities of methanol for 48 h. Light yellow coloured extract was collected. Process was repeated with plant material again and again with fresh methanol till the colour of extract became light. Collected extracts were mixed and methanol was removed to obtain the yellow crude extract, which was kept in an airtight bottle at 4°C temperature.

Antibacterial activity of *C. nurvala*: Antibacterial sensitivity tests were carried out to determine the efficacy of crude extract of bark (*C. nurvala*) against isolated UTI causing bacterial strains. Strains EC1, KL1 and PS1 were seeded uniformly in NAM plates by pour plate technique[5]. 5 mm diam. disc containing 0.3 mg $disc^{-1}$ concentation of crude extract (methanol free) was placed at the four corners of inoculated plates. Inhibition zones were recorded after 48 h incubation at 28° ± 1°C. Control plate did not receive crude extract discs.

Antibiotic sensitivity for bacterial strains: Known concentration of 12 Hi-media antibiotic discs (Table-4) was used in the present study to check the antibiotic sensitivity of UTI pathogens; Same methodology was followed as mentioned above. Antibiotic discs were placed on inoculated NAM plates instead of crude extract discs. Methanol soaked discs were placed at the four corners of inoculated plates that acted as control.

RESULTS AND DISCUSSION

Out of seventy-five urine samples taken in the study, fifty showed the presence of UTI causing bacteria. Three bacterial strains were isolated from infected urine samples and characterized by biochemical test (Table-1) and morphological test on different culture media (Table-2). The isolated pathogenic strains were those of *E. coli, Klebsiella* sp. and *Pseudomonas* sp. In the present study it was recorded that *E. coli, Klebsiella* sp., *Pseudomonas* sp. showed 60%, 24% and 16% colonies in collected urine samples respectively. Colony forming units (cfu) of *E. coli, Klebsiella* sp. and *Pseudomonas* sp. were also recorded as 2.2×10^9, 2.1×10^9 and 2.8×10^9 per mL of urine sample respectively. Earlier Calvin[2] reported that *E. coli* 70%, *Klebsiella* sp. 15%, *Pseudomonas* sp. 15% and *Staphylococcus* sp. 5% and others (3.5%) were presented in patient's urine and caused urinary tract infection in humans. Sherman[6] stated that bacterial count exceeded 10^5 per mL

of urine samples which denotes significant bacterial infection in urine and may cause UTI.

TABLE-1
MORPHOLOGY AND BIOCHEMICAL CHARACTERISTICS OF ISOLATED BACTERIAL STRAINS FROM PATIENTS' URINE

Test	Test organisms		
	E. coli	*Klebsiella* sp.	*Pseudomonas* sp.
Gram staining	–	–	–
Rod shaped	+	+	+
Motility	+	–	+
Spore forming	–	–	–
Fluorescent pigment production	–	–	+
Indole production	+	–	–
Methyl red	+	–	–
Vogues proskauer	–	+	–
Citrate	–	–	–
Oxidase	–	–	+
Catalase	+	+	+
Gas production	+	–	–
Growth at 4°C	–	–	+
Growth at 41°C	–	–	+

TABLE-2
MICROBIAL CHARACTERISTICS OF ISOLATED MICROORGANISMS MORPHOLOGY ON DIFFERENT MEDIA AT 28 ± 1°C AFTER 24 h

Test organism	Media	Observation
Escherichia coli	Nutrient agar	Smooth, greeyish, white or creamy 2–5 mm diam.
	Mac-Conkey	Pink
	Eosin methylene blue	Metallic green
	Chocolate agar	Smooth, greeyish, white or creamy 2–5 mm diam.
	Nutrient broth	Turbidity
Klebsiella sp.	Nutrient agar	Mucoid
	Mac-Conkey	Opaque
	Chocolate agar	Mucoid
	Nutrient broth	Turbidity
Pseudomonas sp.	Nutrient agar	Bluish green
	Mac-Conkey	Fluorescent colonies
	Chocolate agar	Bluish green
	Nutrient broth	Turbidity with greenish colour

Active components from plant bark were extracted in methanol, which is a better organic solvent for the extraction of active component of plant bark[7]. The growth of bacterial strains *E. coli, Klebsiella* sp. and *Pseudomonas* sp. were inhibited by crude extract of *C. nurvala* and zones of inhibition on NAM plates at 28 ± 1°C were recorded as shown in Table-3. Earlier Kirtikar[8] reported that inhibition mainly occurred due to the presence of certain chemical compounds in the crude extract which may be alkaloid, glycosides, essential oil, resins, gum, mucilage etc. that are presented in medicinal plants that define physiological action in human body. On the other hand, known concentration of 12 antibiotic discs showed inhibition zone against *E. coli, Klebsiella* sp. and *Pseudomonas* sp. The maximum zones of inhibition were recorded by amikacin (38 mm diam.), ciprofloxin (40 mm diam.) and ciprofloxin (27.2 mm diam.) against *E. coli,*

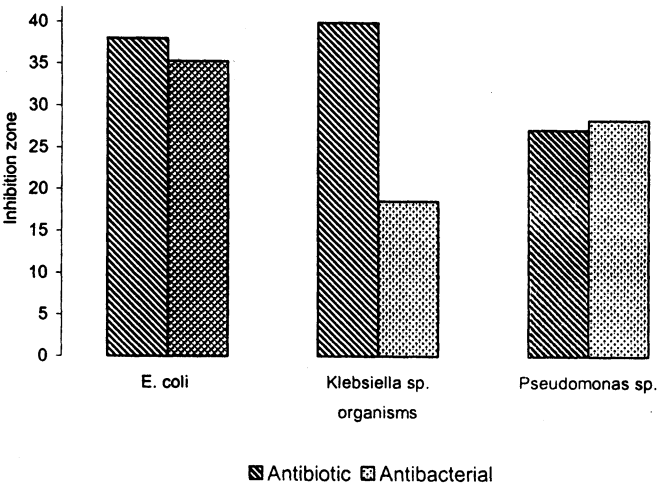

Fig. 1 Comparative representation of antibiotic and crude extract *C. nurvala* (bark) against bacterial strains causing U.T.I.

Klebsiella sp. and *Pseudomonas* sp. on NAM plates at 28 ± 1°C after 24 h incubation respectively (Table-4). The comparative result (Fig. 1) between crude extract and synthetic antibiotic showed that *C. nurvala* was a better alternative of synthetic antibiotics for the treatment of UTI. In case of ayurvedic drugs it is said that they do not have any side effects as well as do not allow the opportunistic microflora in human beings[3]. To overcome these problems ayurvedic therapy was adopted world-wide and a number of ayurvedic drugs are available in the market. Earlier Bauer[9] reported that *E. coli, Klebsiella* sp. *Pseudomonas* sp. and *Proteus* sp. are naturally sensitive to ampicillin, cephalosporin, tetracycline, streptomycin, chloramphenicol, gentamycin and sulfonamide. However, clinical effectiveness of antibiotics is limited owing to development of resistance among bacteria, which causes the establishment of other pathogenic and opportunistic microflora inside the body[10]. *E. coli* showed drug resistance against twelve antibiotics[11]. Bacteria resisted antibiotics after World War II due to the prolonged use of antibiotics[12-14]. Beside this *C. nurvala* plant extract increases the tonocity of the smooth

muscles of the ileum, trachea and uterus in experimental animals and also control the urinary extraction of calcium and the excretory rates of sodium and magnesium, which are involved in the formation of urinary stones[15, 16]. Chaterjee[17] earlier report also revealed that *C. nurvala* plant could be used for the treatment of gastrointestinal disorders and urinary tract infection.

TABLE-3
ANTIBACTERIAL EFFECT OF CRUDE EXTRACT (METHANOL) OF *C. nurvala* BARK AGAINST UTI CAUSING STRAINS

Test organism	Inhibition zone (mm)*
E. coli	35.3 ± 0.013
Klebsiella sp.	18.6 ± 0.017
Pseudomonas sp.	28.4 ± 0.019

*Values are mean of three replicates, ± standard error.

TABLE-4
ANTIBIOTIC SENSITIVITY TEST AGAINST UTI CAUSING ORGANISM ON NAM PLATES AT 28 ± 1°C AFTER 24 h

Antibiotic	Strength (Meg)	Inhibition zone (mm)*		
		E. coli	*Klebsiella* sp.	*Pseudomonas* sp.
Co-Trimoxazole	20	7.5	0.0	12.0
Ceflotaxime	25	7.8	6.0	8.0
Pipcracillin	30	28.0	18.0	18.0
Chloramphenicol	100	4.0	5.0	0.0
Ciprofloxacin	30	3.0	40.0	27.2
Cefizoxine	5	0.0	0.0	0.0
Tetracycline	30	8.0	4.0	0.0
Amplicillin	30	4.5	0.0	0.0
Ofloxacin	5	0.0	0.0	0.0
Gentamycin	10	30.0	20.0	5.0
Amikacin	10	38.0	28.0	9.0
Perfloxacin	10	24.0	26.0	11 .0

*Values are mean of three replicates

On the basis of above result it can be concluded that *C. nurvala* bark can be preferred for the biologically safe treatment of diseases caused by *E. coli, Klebsiella* sp. and *Pseudomonas* sp. instead of synthetic antibiotic treatment.

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