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Antagonistic Activity of *Nocardia brasiliensis* PTCC 1422 Against Isolated Gram-negative Bacteria from Urinary Tract Infections

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The nocardiae are bacteria belonging to the aerobic actinomycetes. They are partially acid fast, Gram-positive, branching filamentous bacteria that are found ubiquitously in soil, fresh water and marine water. Bacterial urinary tract infections are frequent in the outpatient as well as in the nosocomial setting. The common bacteria from UTI_s were isolated from hospital and laboratory samples. The present study was designed to isolate Gram- negative bacteria from urinary tract infections and evaluate *Nocardia brasiliensis* PTCC 1422 antimicrobial activity against pathogenic bacteria. Bacterial isolates were identified as *Acinetobater* spp. and *Pseudomonas aeruginosa*. The cell free supernatants of the *N. brasiliensis* were able to inhibit the growth of all human pathogens (*Acinetobater* spp. and *Pseudomonas aeruginosa*) isolated in this study in Well diffusion method. The isolates also showed very promising activities against multi drug resistant human pathogens.

Key words: *N. brasiliensis*, Urinary Tract Infections, Antagonistic activity.

Actinomycetes are prolific producers of antibiotics and other industrially useful secondary metabolites[2,4,7,14,6]. Nocardiae are aerobic, Gram-positive, non-motile, branching filamentous and catalase positive actinomycetes that are typically acid alcohol- fast at some stages of the growth cycle[1, 3]. Nocardiae are common in soil, populations up to 7.3×10^4/g dry weight have been found in environmental samples from tropical and temperate regions. Most clinically relevant microbial compounds have been natural products or derived from natural products. Among bacteria, actinomycetes have been found to have a unique capacity to produce novel bioactive compounds, notably antibiotics[3]. During the last 40 years, more than 1000 substances and preparations which posses antibiotic properties, i.e., have the capacity to inhibit the growth of and even to destroy various microorganisms, in dilute solutions, have been isolated from culture of an actinomycetes[11].

Urinary tract infection is an infection in the urinary tract. Bacteria are the most common cause of UTI_s[5]. Common and occasional recurrent bacterial illness with an increasing resistance to antimicrobials agents. Antibiotic resistance in UTI is a grow in public health problem in the world[10]. Resistance in Gram- negative bacteria has been increasing, particularly over the last 6 year[8].

This study attempts to evaluate the impact influence of bioactive compounds extracted from *N. brasiliensis* PTCC 1422 on *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated from UTI_s.

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MATERIALS AND METHODS

Isolation of Bacterial strains from UTI_S samples

Samples of urine were collected from UTI_S in North of Iran. *Acinetobacter* spp. and *Pseudomonas aeruginosa* were isolated from patients with urinary tract infections on basis of morphological, cultural and biochemical characteristics according to Berg's Manual Systematic of Bacteriology.

Antibiotic Sensitivity Test (AST)

Antibiotic resistance profile was determined by Kirby Bauer disc diffusion method on Mueller Hinton (MH) agar plates (Hi-media, Mumbai). Discs were consistently tested for efficacy against isolates recommended by National Committee for Clinical Laboratory Standards (NCCLS) as well as others with known antimicrobial susceptibility pattern[9]. The microorganism suspensions used for inoculation were prepared at 10^8cfu (colony forming units)/ml by diluting fresh cultures at McFarland 0.5. Ten several antibiotics (Hi-media) were used for the antibiotic sensitivity test. Standardization of the technique controls variation in results and interpretation was based on comparison of inhibition zones with published criteria for zone diameters[13].

Test organism

The selective microorganisms used for antagonistic activity were *Acinetobacter* spp. and *Pseudomonas aeruginosa* that isolated from UTI_s.

Antagonistic activity

The antimicrobial activity was examined by the agar Well diffusion method. *N. brasiliensis* PTCC 1422 was grown in 50 ml of starch casein broth by submerged culture containing in 250 ml flasks by incubating at 28 °C in a shaker (150 rpm) for 7 days and centrifuged at 4000 rpm for 10 min and the clear supernatant broth samples were tested for their antagonistic activity against the isolated pathogens by agar well diffusion method[11,12]. Wells of 6 mm diameter were prepared in the nutrient agar plates. Isolated pathogenic bacteria were swabbed on to the nutrient agar surface[11] and the wells were filled with the 70 µl of culture supernatant and the diameter of inhibition zones were measured after incubation for 24 h at 37°C[2].

GC- MS analysis

In this technique the Gas Chromatography and Mass Spectrometry was analyzed by GC-MS electron impact ionization (EI) method on GC-8000 gas chromatograph (FISONS Instruments). Compound identification was done by comparing the NIST (National Institute of Standards and Technology- Chemistry web book by WILEY) library data of the peaks with those reported in literature[15].

RESULTS AND DISCUSSION

Isolation and screening of bacteria from UTI_S

Acinetobacter spp. and *P. aeruginosa* were isolated from UTI_s samples following standard protocols. The best strain was characterized by biochemical, morphological and physiological tests. According to Bergey, s manual of determinative bacteriology and the laboratory manual for identification of bacteria, the isolates were identified are shown in the Tables 1 and 2 (6).

Antibiotic Sensitivity Test (AST)

The isolates with high antibiotic resistance were identified and tested against 10 antibiotics from different groups. The isolates showed high level of resistance to multiple antibiotics and were resistant to more antibiotics. Resistance level was low to Tetracycline as compared to other antibiotics of this group (Fig. 1).

Determination of Antimicrobial activity

N. brasiliensis PTCC 1422 showed potential antagonistic activity against Acinetobacter spp. and *P. aeruginosa* in the present study. The observed inhibition zone diameters are given in Figure 2. The inhibition zone diameters (mm) obtained by experimental trials were found to be in the range of 8-12 mm.

GC- MS analysis

In GC-MS analysis totally 17 compounds were identified. The Mass Spectrometry indexes of the major peaks are shown in the Figure 3. The GC-MS analysis shown that 2 major peaks. The highest peak area %50.56 by Phthalic acid (Di-(2-ethylhexyl)phthalate) with retention time 24.26 min and %11.62 obtained by Decanedioic acid with retention time 4.30 min(Table 3).

N. brasiliensis PTCC 1422 exhibited antibacterial activity against isolates and showed promising activity in vitro condition. Similar study was carried out in Egypt by Nermeen A. El- sersy

Table 1. Biochemical characters of *Acinetobacter* spp

Characteristics	Gram stain	Motility	Oxidase	Catalase	Urease	OF medium
Acinetobacter spp.	-	-	-	+	-	+

+ positive; - negative

Table 2. Biochemical characters of *Pseudomonas aeruginosa*

Characteristics	Gram stain	Motility	Indol	SH_2	OF	Oxidase	Catalase	Citrate	MR	VP
Pseudomonas aeruginosa	-	+	-	-	+	+	+	+	-	-

+ Positive; - negative

Table 3. Concentrations of determined compounds in *N. brasiliensis* by GC-MS method.

No	R_t(min)	Name of Compound	%
1	3.38	Brasilicardin A	2.42
2	4.00	2,6-piperdionemonoxime	1.55
3	4.30	Decanedioic acid	11.62
4	11.31	Hexadecanoic acid	0.95
5	12.68	Cyclopentaneundcanoic acid	1.16
6	13.16	Phenyl ethyl alcohol	1.92
7	14.34	1- phenyl but- 3- ene- 2- ol	2.59
8	16.81	Di -Butyl Phthalate	1.15
9	17.48	Beta – 1-arabinopyranoside methyl	1.23
10	17.94	Palmitic Acid	3.57
11	19.56	Terpenoid	2.99
12	20.23	Antimycin	2.22
13	22.71	Eicosane	1.75
14	22.74	1,2- benzenedicarboxylic acid 3- nitro	1.38
15	23.69	1- eicosanol	1.39
16	24.26	Di-(2-ethylhexyl) Phthalate	50.56

whose studied the antagonistic effect of marine *N. brasiliensis* against the fish pathogen *Vibrio damsela*. *N. brasiliensis* produced a compound that showed the largest inhibition zone and highest activity against the tested (*Acinetobacter* spp. and *P. aeruginosa)* organism and the inhibition zones were in the range of 10 to 12 mm[11].

Thus in our present study the isolates

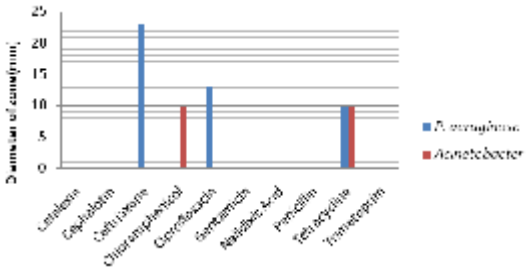

Fig. 1. Sensitivity of isolated bacteria from UTI_S to antibiotics in mm

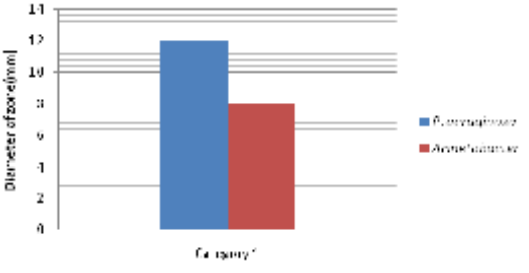

Fig. 2. Antimicrobial activity of *N. brasiliensis* against isolated bacteria fromUTI_S in mm

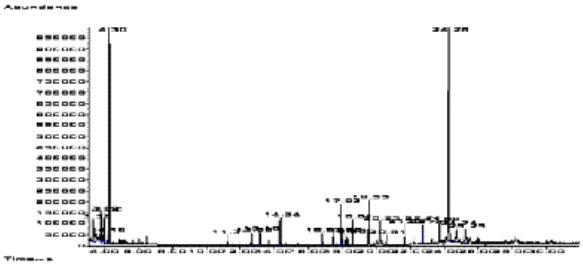

Fig. 3. Spectrum of determined compounds in*N.brasiliensis* by GC-MS method

were identified as *Acinetobacter* spp. and *P. aeruginosa*. The cell free supernatant of the *N. Brasiliensis* PTCC 1422 was subjected to chemical analysis using chromatographic system including GC-MS. It was able to inhibit the growth of isolated bacteria from UTI_s used in this study include *Acinetobacter*s pp.(8 mm) and *P. aeruginosa* (12 mm) in well diffusion method. Present study clear indicates antagonistic activity of *N. brasiliensis* PTCC 1422 against isolated bacteria from urinary tract infections (*Acinetobacter* and *P. aeruginosa*) in vitro condition.

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