Antimicrobial Effect of *Cyperus rotundus* on Multiple Drug Resistant *Pseudomonas aeruginosa* Strains

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ABSTRACT

***Background*:** Medicinal use of plants dates long back in the history of the human beings and has recently gained a great deal of importance in treating different diseases. Due to the importance of *Pseudomonas aeroginosa* strains as agents responsible for common secondary infections and their resistance to antibiotics and disinfectants, the present study aimed at investigating the antimicrobial effects of the plant *Cyperus rotundus* on multiple drug resistant (MDR) *P. aeruginosa* strains.

***Methods*:** Ethanolic extracts of *Cyperus rotundus* tuber were prepared by maceration. The antimicrobial effect of these extracts on *P. aeruginosa* isolates (ie., sensitive to antibiotics and multiple drug resistant strains), isolated from clinical and soil samples, was determined by both disk diffusion and broth microdilution methods..

***Results*:** It was revealed that ethanolic extract concentrations of *Cyperus rotundus* tuber higher than 0.1 mg/ml suppresses the growth of all antibiotic sensitive and resistant *P. aeruginosa* strains which were used in this study.

***Conclusion*:** It is concluded that *Cyperus rotundus* possesses antimicrobial properties and thus can be used in treating multidrug resistant *P. aeruginosa*-induced wounds and infections.

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Introduction

Pseudomonas aeruginosa is an aerobic, opportunistic, Gram negative bacterium with lipopolysaccharide, polar flagella and pili (1, 2). The bacterium is distributed widely in nature and acts as an infecting agent in patients inflicted with immune deficiency, neutropenic, burns and catheter. It is also the most common agent in infecting the respiratory, urogenital, digestive and the central nervous systems, as well as bone and cartilage tissues, skin and soft tissue, bacteremia, septicemia and eye and ear infections (3, 4, 5).

Treatment of *Pseudomonas aeruginosa* infected patients, in particular those infected with the antibiotic resistant strains, is not an easy task (6). Following inappropriate experimental treatments, sensitive microorganisms also turn resistant, a process requiring the induction of the antibiotic inactivating enzymes or mutations in genes encoding outer membrane porins, or plasmid transfer. Beta lactamase production is one of the most important mechanisms of bacterial resistance, and the number of beta-lactamase producing bacteria are on the rise due to mutations (7, 8).

Plants have served as medicine since the ancient times (9). Similar to antibiotics, the antimicrobial effects of medicinal plants have gained a great deal of attention (10, 11). Use of medicinal plants in the past several decades has increasingly turned popular such that a great number of the available medicines are of plant origin (12, 13), and medicinal plants are currently being used for the treatment of many diseases including fungal infections (14). The aim of the present study was to determine the contamination degree of tap waters with *P. aeruginosa*, *L. pneumophila*, and *E. coli* in all cities in Guilan province, Iran.

Cyperus rotundus, also known as nut grass, as a member of the Cyperaceae family is distributed in humid, marshy, warm and moderate environments including North Iran with a local name of "*Teplagh*". It is a perennial plant with 20-40 cm height, long roots with rhizomes and a black shelled tuber with a white and odorous interior. Leaves are numerous, small, linear, dark green, and spikelets are reddish brown (15, 16, 17). The rhizome contains essential oil, saponins, vitamin C, polyphenol and flavonol glycoside (18, 19). *Cyperus rotundus* has had medicinal use in gastrointestinal bloating, stomach burning, stomach ulcers, menstrual periods, kidney problems, headaches and liquid mouthwash (20, 21, 22).

Due to the increasing importance of medicinal plants in herbal medicine and their low rate of side effects, and the significant hospital infections caused by *Pseudomonas aeruginosa* and its resistance to most antibiotics, this study aimed to investigate the antimicrobial effects of the ethanolic extract of *Cyperus rotundus* on both sensitive and multidrug resistant *Pseudomonas aeruginosa* strains.

Material and method

Isolation and purification of Pathogenic microorganisms

To isolate and purify *Pseudomonas aeruginosa* strains, including; 40 clinical samples from patient wounds, hospitalized at Motahari Hospital, Tehran, Iran. Also 50 soil samples from Eznova-Behnemir region Mazandaran-Iran were prepared in sterile tubes and transferred to the laboratory under sterile conditions. Serial dilutions of 10^{-1}-10^{-10} were prepared from samples and plated on citrimide medium in duplicates. Plates were incubated at 37 ºC for 48 h and bacterial colonies were subjected to both macroscopic and microscopic examinations. *Pseudomonas aeruginosa* strain identification was performed by Gram staining, oxidase, catalase, pigmentation, OF, citrate, urease, arginine dehydrolase, lysine and ornithine decarboxylase tests, interaction in TSI medium, and growth at 42 ºC (23).

Cyperus rotundus tuber extract preparation

Cyperus rotundus tubers were collected from the agricultural lands of Aznava-Behnamir in Mazandaran province of Iran. Extracts were

prepared by maceration or wetting by ethanol solution. Tubers were dried in a closed environment with ventilation, and then powdered. A total of 200 grams of the powder was added to 100 ml of ethanol and incubated for 48 h, and then filtered through a sterile paper filter. This filtered extract was then dried by incubation at 40 ºC.

Strain sensitivity to antibiotics

Initially, microbial suspensions of the bacteria with a turbidity of half a McFarlen (1.5×10^8) were prepared according to CLSI (The Clinical and Laboratory Standards Institute). Samples of microbial suspensions were grown on Muller Hinton plates using a sterile swap. Bacterial sensitivity to antibiotics Amikacin (30 µg), Ciprofloxacin (5 µg), Piperacillin (100 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefepime (30 µg), Imipenem (10 µg), Meropenem (10 µg) was tested using antibiotic discs (Padtan Teb, Iran) (Kirby bauer) (24). Following incubation at 37 ºC for 18-24 h, the diameter of the zone of inhibition around the disc was measured. Bacterial strain sensitivity or resistance to antibiotics was determined according to the standard table of CLSI.

Antimicrobial activity of the tuber extract

Strain sensitivity was determined by both disk diffusion and broth microdilution methods. Initially, suspensions of 0.5 McFarlen (1.5×10^{-8} CFU/ml) were prepared (25).

In the disc diffusion method, using a sterile swap, suspensions were grown on Muller Hinton agar medium. Sterile blank discs were placed in plates containing different concentrations of the tuber extract and then placed on agar plates containing microorganisms. These plates were incubated at 37 ºC for 24 h, and then subjected to measurements of the zone of growth inhibition (17).

In the broth microdilution method, tubes containing Muller Hinton broth medium containing 0.1-20 mg/ml of the tuber extract to a total volume of 1 ml were prepared and inoculated with 100 µl of 0.5 McFarlen suspension (26). A solution of 0.1 mg/ml of the extract was also included as negative control. MIC values were determined after 24 h of incubation at 37 ºC (27).

Results

In total a collection of 84 *Pseudomonas aeroginosa* strains were isolated from both clinical (50 strains) and soil samples (34 strains) and identified by biochemical tests. Of these, 21 strains were multidrug resistant (19 from clinical and 2 from soil samples) (Table 1).

Table 1. Results of the antibiogram test in multiple drug resistant *Pseudomonas aeroginosa* strains.

Antibiotic	# of Resistant Strains	# of Resistant Clinical Strains	# of Resistant Soil Strains
Total number of resistant strains	21	19	2
Amikacin	15	15	0
Ciprofloxacin	10	10	0
Piperacillin	13	13	0
Piperacillin	21	19	2
Ceftazidime	20	19	1
Cefepime	20	19	1
Imipenem	21	19	2
Meropenem	21	19	2

Strain sensitivity to Cyperus rotundus tuber extract

In the blank disc method, zone of growth inhibition appeared at a minimum inhibitory concentration of 25 mg/ml for all bacterial strains (Table 2).

Table 2. Blank disc results on the inhibitory effect of *Cyperus rotundus* extract on *Pseudomona aeroginosa*.

Microorganism	Diameter of zone of growth inhibition (mm) at 25 mg/ml of the extract
Antibiotic sensitive *Pseudomonas aeroginosa* strains	15
Multiple drug resistant *Pseudomonas aeroginosa* strains	15

In the broth microdillution method, the MIC value for all bacterial strains was at a concentration of 0.1 mg/ml (Table 3).

Table 3. MIC values of the ethanolic extract of *Cyperus rotundus* on the growth of *Pseudomonas aeroginosa*.

Microorganism	MIC value (mg/ml)
Antibiotic sensitive *Pseudomonas aeroginosa* strains	0.1
Multiple drug resistant *Pseudomonas aeroginosa* strains	0.1

Discussion

In the present study, 84 *Pseudomonas aeroginosa* strains resistant and sensitive to antibiotics, prepared from both clinical and soil samples, were isolated using citrimide agar medium, and identified based on phenotypic and biochemical analyses. These bacterial strains were then treated with the ethanolic extract of *Cyperus rotundus* tuber. It was revealed that the tuber extract possesses an antimicrobial effect, such that the antibiotic resistant strains did not show any resistance to the extract, and the MIC value for all the strains was determined to be 0.1 mg/ml.

Cyperus rotundus tuber extract has been shown to possess antimicrobial activity (28). While, its inhibitory effect against *Streptococcus pyogenes* growth was demonstrated by Mehta et al. (29), the whole plant extract has been shown to be ineffective against strains of *E. coli*, *Pseudomonas aeroginosa* and *Salmonella typhi* (30). A MIC value of 12.5 mg/ml has indeed been determined for certain bacterial strains treated with the rhizomes oil (17), whereas using the disc diffusion method, zone of growth inhibition was revealed in *Pseudomonas aeruginosa and Staphylococcus aureus* treated with the ethanolic extract of the plant (31). In a separate study, using the agar disk diffusion method, the ethanolic extract of *Cyperus rotundus* revealed a zone of growth inhibition for *E. coli* and *C. albicans* (32).

Conclusion

In conclusion, *Cyperus rotundus* extract possesses an antimicrobial effect and inhibits the growth of resistant *Pseudomonas aeroginosa* and thus maybe considered as an effective agent in treating patients infected with MDR strains of *Pseudomonas aeroginosa*. Considering the increasing trend in the use of antibiotics along with the major clinical and public health problems associated with antibiotic resistance, plant based antimicrobial agents, such as those contained within *Cyperus rotundus* extract, will be of great use in both preventing and curing *Pseudomonas aeroginosa* related infections.

Conflict of interests

No conflict of interests is declared.

Financial disclosure

Authors declare no financial disclosures.

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