The Survey of *Withani somnifera* Extraction against Resistant Strains of *Pseudomonas aeruginosa* Bacteria to Selective Antibiotics

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ABSTRACT

***Introduction*:** Due to more resistance of pathogenic bacteria to new and current antibiotics researchers are looking to find the agents of herbal with antimicrobial activities in order to replace chemical drugs.

***Methods*:** The herbal extract of *Withani somnifera* was done by using a rotary vacuum, 20 strains of *Pseudomonas aeruginosa* were isolated from urinary infections hospitalized patients in city of Zabol hospital. The MIC *Withani somnifera* were determined by dilution method in various concentrations. Sensitivity of strains to multiple antibiotics was evaluated by standard disk diffusion Kirby-Bauer.

***Results*:** The result showed that *P. aeruginosa* were resistance to 4 of the agents including ampicillin (85%), nitrofurantoin (65%), nalidixic acid (65%), ciprofloxacin (15%) and for 5 strains of Pseudomonas showed MIC with activity of 100 ppm.

***Conclusion*:** This study has suggested the effect of winter cherry extract on *P. aeruginosa* in the in vitro assay. It s effectiveness of on in vivo system can be examined in future.

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Introduction

More increasing resistance of drugs among bacteria caused researchers to pay more attention in finding new ways to prevent bacterial resistance to drugs. The opinion of therapeutic capabilities of some plants returned to ancient times. Some of the natural products, which we use them on daily basis, contain antimicrobial substances (2). *Pseudomonas aeruginosa* are rod-shape gram negative bacteria. They are opportunist bacteria that can cause sepsis, endocarditis, skin, ears, urinary and eyes infections (3). *P. aeruginosa* is the second most current pathogenic bacteria in surgical operations, and the third agents of hospital infections after *E. coli* and *Staphylococcus aureus*, and they are the cause of 10% of hospitalized infections (4) which have become multiple drug resistance. Because of having high resistance to antibiotics, treatments of infections that are caused by these bacteria are very difficult. Winter cherry is an evergreen (perennial) plant, like bushes with 30 to 100 cm high. It grows all over Pakistan and southwest of India and Afghanistan. In Iran, the growth zone is at Baluchistan of Sistan and Baluchistan province. The natural growth zone in Baluchistan is Saravan, Khash and the mountain of Bamposht (5). Winter cherry has an anti-inflammation (6), anti-oxidant (7), enforcement memory (8) and reducing pain (9) activities. The purpose of this study was to investigate the ethanol extract of winter cherry on the isolated strains of *P. aeruginosa* from clinical samples.

Materials and Methods

Bacteria strains

All 20 strains *P. aeruginosa* (isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections**.** Cultures were grown on nutrient agar. Pseudomonas identification was carried out using gram stain, catalase, oxidizes, glucose tests, oxidation fermentation and triple sugar iron (10).

Agar disk diffusion assay

Resistance to tetracycline was tested by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocols. Antibiotic disks were obtained from the Patan ted Co. (Tehran, Iran). *P. aeruginosa* isolates were evaluated based on the size of the zones of inhibition and classified as susceptible (S), intermediate resistant (I) or resistant (R) according to the CLSI criteria (11).

Plant material

The leaves of *W. somnifera* were collected from Iran and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of extracts

Twenty grams of selected fresh leaf materials were macerated with 60 mL of ethanol 95%, in a grinding machine for about 10 to 15 minutes for separating the extract phases for one day (shaking occasionally with a shaker). The supernatant was filtered through Whatman No. 1 filters paper. The extracts were preserved aseptically at 5°C for further use.

Minimum inhibitory concentration (MIC) of plant extracts

The broth micro dilution method was used to determine MIC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v).

Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 12.5 ppm to 200ppm. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth was added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hrs. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

Results

The results of this study showed that isolated pseudomonas were respectively resistant to antibiotics: However, overall *P. aeruginosa* were resistance to 4 of the agents including ampicillin (85%), nitrofurantoin (65%), nalidixic acid (65%), ciprofloxacin (15%) (Table1) and the result of plant extraction showed that the most MIC was 100 ppm concentration, and 5 strains of pseudomonas were inhibited (Table 2).

Table 1. Percentage of antimicrobial susceptibility of 20 strains of *P. aeruginosa*

	Am	Fm	NA	CP
S	0	60	10	60
I	15	20	25	25
R	85	20	65	15

CP= ciprofloxacin, NA= nalidixic acid,
Fm= nitrofurantoin, AM= ampicillin
S= Sensitive, I= Intermediate, R= Resistant

Table 2: MIC of *Withani somnifera* extract on drug resistant bacteria strains

Bacterial cod	Am	Fm	NA	CP	The least MIC(ppm)
1	R	R	R	S	50
2	R	S	S	S	50
3	R	S	R	S	50
4	R	S	R	I	50
5	R	S	I	R	100
6	R	S	R	R	100
7	R	R	R	S	50
8	I	I	I	I	50
9	I	S	R	S	50
10	R	R	R	S	100
11	R	S	I	I	100
12	R	R	R	S	100
13	R	S	R	S	50
14	R	S	S	R	50
15	R	I	I	I	50
16	I	I	I	I	50
17	R	S	R	S	50
18	R	S	R	S	50
19	R	S	R	S	50
20	R	I	R	S	25

Gm= gentamicin, CAZ= ceftazidime, SXT= sulfamethoxazol-trimethoprim, CP= ciprofloxacin, NA= nalidixic acid, Fm= nitrofurantoin, AM= ampicillin

Discussion

The results of this study showed that isolated *Pseudomonas aeruginosa* were resistant to antibiotics ampicillin (85%), nitrofurantoin (65%), nalidixic acid (65%), and ciprofloxacin (15%). In a study by loureiro and colleagues in a hospital in Brazil it was reported that the isolated pseudomonas were resistant: chloramphenicol (96/29%), ceftriaxone (96/27%), tetracycline (75/21%), gentamicin and amikacin (10/3%) (12).

In Haleem's study results showed that the isolated pseudomonas from environment were entirely resistant to penicillin, ampicillin, tetracycline, erythromycin, chloramphenicol, ceftriaxone and also were sensitive to the tikarcillin (78%) and piperacillin (71%) (13). the results of Eslami *et al* study showed that isolated pseudomonas were resistant to ceftriaxone (37%), ceftriaxone (100%), gentamicin (44%), imipenem (12%) and ampicillin (84%) (14). and also the results of Rahimi *et al* study showed that isolated pseudomonas were resistant to imipenem (35%), meropenem (35%), gentamicin (14%), amikacin (9%), ciprofloxacin (15%) and ceftazidime (23%) (15). In a study that was done in Kermanshah (Iran) showed isolated pseudomonas were resistant to amikacin (38%), azlocillin (52%), ceftazidime (50%), ciprofloxacin (38%), gentamicin (52%) and tobramycin (46%) (16). The result of plant extraction in this study showed that the most MIC was within 100ppm concentration, and 5 strains of pseudomonas were completely inhibited. The results in Singariya study showed that ethanol extracts of winter cherry can inhibit *Proteus mirabilis*, *Klebsiella pnemoniae* and *Agerobacterium tumefaciens* by different inhibitory zones (14/33±0/25, 0/83±0/27 and 7/5±0/64) (17). Results in Jaina study showed the method extracts of root winter cherry could inhibit *E. coil*, *P. aeruginosa*, *S. aureus* and *S. mutans* by inhibitory zone 38,15,36,38 mm, respectively (18).
In Singh study the MIC to *N. steroids* and *S. pyogenes* were 31 and 62µg/ml, respectively (19).

Conclusion

Considering all the evidence, the positive results from this study suggest that the herbal compounds of *Withani somnifera* could be an alternative and suitable replacement for chemical drugs.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of interest

None declared conflicts of interest.

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