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Investigating Antimicrobial Effects of *Tecomella undulata* Ethanolic Extract on Antibiotic Resistant *Acinetobacter baumannii*

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ABSTRACT

Considering the increasing resistance of bacteria to antibiotics and the presence of antibacterial agents in plants, in this study, the antimicrobial activity of *Tecomella undulata* ethanolic extract on antibiotic resistance *Acinetobacter baumannii* has been studied.

Materials and methods: The leaves of *Tecomella undulata* were collected from Saravan city and extracted by rotary machine. *Acinetobacter bomanii* strains were collected from urine specimens of Imam Khomeini and Ali ibn Abi Talib Hospitals. Minimum inhibitory concentration and minimum bactericidal concentration were determined by micro dilution method.

Results: The results of this study showed that the resistance of the strains was to amoxiclavanic (10%), ampicillin (20%), gentamicin (0%), ceftazidime (0%) and nitromicin (0%) antibiotics.

The results of this study showed that the lowest inhibitory concentration of *Tecomella undulata* is 0.62 mg/ml, which inhibits 6 strains in this concentration, while the highest inhibitory concentration is 5 mg/ml, which inhibited 3 strains in this concentration.

Discussion: By considering the results, obtained and increasing resistance of bacteria to chemical antibiotics, it is suggested that bacterial compositions of this plant can be used to treat bacteria.

Keywords: *Tecomella undulata*, Antimicrobial activity, *Acinetobacter baumannii*

INTRODUCTION

Acinetobacter baumannii is one of the most important pathogens in health centers that cause many infections including bacteremia, pneumonia, meningitis, urinary tract infections and ulcers. The ability to survive under various environmental conditions has made this pathogen one of the most common causes of infection in health centers [1].

Tecomella undulata is an Antarctic pink or embroidered pomegranate, an almost evergreen tree that runs in the southern regions of the country such as Bushehr, Fars and Hormozgan.

In addition, its distribution in Afghanistan, West Pakistan and southeastern Arabia has been recorded. Due to medicinal properties, this plant has been considered as a good treatment [2]. Flavonoid compounds, phytosterol, flavonol, fatty acids and terpenoses have been identified in various parts of the plant [3]. It has anti-inflammatory, antimicrobial and anti-oxidant activity [3,4].

This plant is useful in draining urine and enlarging the spleen. The skin of the young shoots of the plant is used for the treatment of syphilis [5]. The purpose of this study was to evaluate the antimicrobial activity of the ethanolic extract of *Tecomella undulata* on the antibiotic-resistant acintobacter bomanii in Zabol.

MATERIALS AND METHODS

In this study 20 isolates of *Acintobacter baumanni* from infected patients in Imam Khomeini and Ali ibn Abi Talib Hospitals in Zabol were investigated.

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Laboratory procedures

The clinical specimens were cultured on the McConky Agar and Blood Agar medium then plates were incubated at 37°C for 24-24 h. An oxidase test was performed in case of growth after gram staining and observation of cocci and gram negative diplucoxia. In the next step, by using biochemical tests, cultured on McConky agar and incubated at 37°C and 42°C, then citrate and moving test were performed on the of media containing glucose.

Determination of antibiotic susceptibility

Determination of susceptibility was done by standard Disc diffusion agar. For testing, bacterial colonies, 0.5 µM MacFarland suspensions were prepared and well-spread over the Muller Hinton Agar medium. Then Antibiotic discs were placed at standard spacing. After 24 h incubation at 37°C, the non-growth diameter for each antibiotic was measured. The results were recorded for each antibiotic according to the relevant instructions as sensitive, intermediate and resistant.

Preparation of ethanolic extract

Tecomella undulata collected from Saravan city and dried. To prepare the ethanolic extract, 10 g of dried powder were placed inside half-liter erlenn containing 100 ml of 96% ethanol (to prepare the ethanolic extract). The contents of the erlenn were mixed at room temperature for 24 h by shaker machine (Iran) at 130 rpm and then filtered with Wattman No. 2 paper. Solvent separation from the extract was performed by a rotary machine (Heidolph-Germany) with the aid of a vacuum pump (vacuum distillation). The extracts were weighed and then solved in DMSO solvent. The extract was stored in a refrigerator until use in antimicrobial experiments at 4°C.

Determination of susceptibility of bacterial strains to different extracts of pomegranate plant

Determination of susceptibility of bacterial strains to plant extracts was performed using a dilution method in well. Six wells were created in a solid culture medium and 100 µl of each well was added to the nutrient medium of Muller Hinton (MHB). Then, to the first well, 100 ml of diluted solution of the extracts of plants was added and after mixing 100 µl of the first well, added to the second well, and this was done until the last well. From the final well, 100 µl of the medium was extracted and 10 µl of the microbial suspension containing 10^7 µg/ml which was equal to 0.5 McFarland added and incubated at 37°C for 24 h. The first pill that was prevented bacterial growth after placing in the incubator was considered as the minimum inhibitory concentration. In order to ensure, 10 µl from transparent wells were transferred to the Muller Hinton Agar medium and after 24 h the first concentration that could eliminate 99.9% of the bacteria was considered as the minimum bactericidal concentration.

RESULTS

The results of this study showed that the strains were resistant to amoxicklavanic antibiotics (10%), ampicillin (20%), gentamicin (0%), ceftazidime (0%) and erythromycin (0%) **(Table 1)**.

Table 1. Antibiotic pattern.

Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
AMC	1 (10%)	1 (10%)	8 (80%)
AM	2 (20%)	3 (30%)	5 (50%)
GM	0 (0%)	0 (0%)	0 (100%)
CZ	0 (0%)	0 (0%)	0 (100%)
AZM	0 (0%)	0 (0%)	0 (100%)

The results of this study showed that the lowest inhibitory concentration of *Tecomella undulata* was 0.62 mg/ml, of which 6 strains were inhibited at this concentration, while the highest inhibitory concentration was 5 mg/ml which three strains have been inhibited in this concentration. The highest bactericidal concentration was 10 mg/ml, which 2 strains were eliminated at this concentration, while the lowest bactericidal concentration was 1.25 mg/ml **(Table 2)**.

Table 2. MIC and MBC extract plant.

Strain bacteria	MIC	MBC
1	2/5	5
2	5	10
3	2/5	5
4	0/62	1/25
5	1/25	2/5
6	5	10
7	2/5	5
8	0/62	1/25
9	0/62	1/25
10	0/62	1/25
11	2/5	5
12	0/62	1/25
13	1/25	2/5
14	0/62	1/25
15	2/5	5
16	1/25	2/5
17	5	5
18	2/5	5
19	1/25	2/5
20	2/5	5

DISCUSSION

The results of this study showed that the strains were resistant to amoxicklalanic antibiotics (10%), ampicillin (20%), gentamicin (0%), ceftazidime (0%) and nitromicin (0%) [6].

The results of this study showed that the highest resistance was to ceftriaxone, ciprofloxacin and cefotaxime, which was 99%, observed in Angoti et al. [7], who investigated the drug resistance of *Acinetobacter baumannii* strains in Imam Reza Hospital. The percentage of isolates resistance to ampicemin, amikacin and ciprofloxacin was 73.3%, 38.3% and 93.3% in the E-test method, respectively.

The results of Rastu and Bad [8], who investigated the *Acinetobacter baumannii* resistance pattern in Shariati Hospital in Tehran, showed that the highest sensitivity was to ciprofloxacin (91%), cotrimoxazole (57.5%) and the highest resistance rate was to ceftriaxone (98.4%).

In the study of Nah et al. [9], who evaluated the resistance level of *Acinetobacter baumannii* strains in Tehran city, the results showed that all isolates of *Acinetobacter baumannii* were resistant to ceftizoxime, cefoprazone, ceftazidime, tricarcelin, clavulanic acid, cefotaxime, aztreonam, Moropenem, cefixim, ceftriaxone, carbenicillin and ticarcylin, but all isolates were sensitive to cholestin.

The results of Ahmadikiya et al. [10], who investigated antibiotic resistance of *Acinetobacter baumannii* in Kerman, showed a resistance rate to antibiotics such as cefotaxime (100%), ceftazidime (98.9%), cefipime (100%), aztreonam (98.9%), ampicemin (97.9%), meropenem (97.9%), gentamicin (96.8%), amikacin (98.9%), ciprofloxacin (97.9%), ciprofloxacin (97.9%) and tetracycline (90.5%).

In the study of Simhon et al. [11], Sensitivity to ampicemin was 98.1% in 1990 but reduced to 64.1% in 2000 and the sensitivity to ciprofloxacin decreased from 50.5% to 13.1%. In a study by Boromand et al. [12] in Tehran, 53.4% of the

samples were resistant to ciprofloxacin and 24.6% resistant to ampicemi.

In the study by Henwood et al. [13], 46% of the isolates were resistant to ciprofloxacin and 2% of the samples were resistant to ampicemin.

The results of Zhao study [14], who investigated the resistant pattern of *Acinetobacter baumannii* showed a resistance rate to ampicillin (78.5%), cefazolin (78.5%), imipenem (92.3%), gentamicin (87.7%) and ampicillin resistance, Ceftazidime (92.3%), aztreonam (92.2%), ciprofloxacin (98.5%) and tobramycin (81.5%).

The results of Rahbar et al. [15] study on prevalence of antibiotic resistant showed that resistance to ceftriaxone (90.9%), piperacillin (90.9%), ceftazidime (84.1%), amikacin (2/85%) and ciprofloxacin (90.9%).

In the study of Uwingabiye et al. [16], resistance to antibiotics such as ciprofloxacin, ceftazidime, piperacillin-tazobactam, imipenin, amikacin, tobramycin, dabylmezin, rifampin, colistin were 87%, 86%, 79%, 76%, 52%, 43%, 33%, 32% and 1.7%, respectively.

In recent decades, the research priority has fallen down to make new and effective drugs; this is despite the fact that the world faces pathogens with drug resistance. Another concern in this regard is the cost of treating drug-resistant infections due to the higher cost of new drugs and the long time treatment of antibiotic-resistant infections than susceptible bacterial infection, which doubles the importance of finding a new method for treatment [17].

In the study of Abhishek et al. [18], the minimum inhibitory concentration of methanol extract against *B. subtilis*, *E. fecalis*, *E. coli*, *K. pneumonia*, *M. luteus*, *P. vulgaris* and *P. aeruginosa* was equal to 4-0.01-0.1-2.0 mg/ml, respectively. In Thanawala and Jolly [19], Inhibition diameter of Acetonic extract of *Tecomella undulata* was compared to *Bacillus subtilis* (17 mm) and *Staphylococcus aureus* (10 mm), while the inhibitory diameter of alcoholic extract of *Tecomella undulata* against the *Escherichia coli* was 9 mm.

CONCLUSION

Considering the obtained results and the increasing resistance of bacteria to chemical antibiotics, it is suggested that, to conduct more studies on antibacterial compounds of this plant in treatment of bacterial infections.

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