

## Investigating Antimicrobial Effects of *Tecomellaundulate* ethanolic Extract on Antibiotic Resistant *Acinetobacterbomanii*

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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 *Tecomellaundulata* ethanolic extract on antibiotic resistance *Acinetobacter bomanii* has been studied.

**Materials and Methods:** The leaves of *Tecomellaundulata* 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 microdilution 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 *Tecomellaundulata* is 0.62 mg / ml, which inhibits 6 strains in this concentration, while the highest inhibitory concentration is 5 mg / ml L, which inhibited 3 strains in this concentration.

**Discuss:** 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:** *Tecomellaundulata*, Antimicrobial Activity, *Acinetobacterbomanii*

### INTRODUCTION

*Acinetobacter bomanii* 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). *Tecomellaundulata* 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 (Mozaffarian 2009). 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 *Tecomellaundulata* on the antibiotic-resistant *acinetobacterbomanii* in Zabol.

### MATERIALS AND METHODS

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

#### Laboratory Procedures

The clinical specimens were cultured on the McCanky 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

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after gram staining and observation of cocci and gram negative diplococci. In the next step, by using biochemical tests, cultured on McConkey 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, 0.5µM MacFarland suspension were prepared and well spreaded over the Muller Hinton Agar medium. Then Antibiotic discs were placed at standard spacing. After 24 hour 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

*Tecomellaundulata* collected from Saravan city and dried. To prepare the ethanolic extract, 10 grams of dried powder were placed inside half-liter erlenmeyer flask containing 100 ml of 96% ethanol (to prepare the ethanolic extract). The contents of the erlenmeyer flask were mixed at room temperature for 24 hours by shaker machine (Iran) at 130 rpm, and then filtered with Whatman 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 dissolved 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 100µl of each well was added to the nutrient medium of Muller Hinton (MHB).

Then, to the first well, 100 µl of diluted solution of the extracts of plants was added and after mixing 100µl of the first well, added to the second well, and this was done until the last well. From the final well, 100µl of the medium was extracted and 10µl of the microbial suspension containing 107µg / ml which was equal to 0.5 McFarland and incubated at

37 ° C for 24 hours. The first well that was prevented bacterial growth after placing in the incubator was considered as the minimum inhibitory concentration.

In order to ensure, 10µl from transparent wells were transferred to the Muller Hinton Agar medium, and after 24 hours 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 amoxiclavanic antibiotics (10%), ampicillin (20%), gentamicin (0%), ceftazidime (0%) and erythromycin 0% Table 1.

**Table1.** Antibiotic pattern

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

**Table2.** 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

The results of this study showed that the lowest inhibitory concentration of *Tecomellaundulata* 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).

## **DISCUSSION**

The results of this study showed that the strains were resistant to amoxiclavanic antibiotics (10%), ampicillin (20%), gentamicin (0%), ceftazidime (0%) and nitrocin (0%). The results of this study showed that the highest resistance was to ceftriaxone, ciprofloxacin and cefotaxime, which was 99 %, observed in Engoti et al., who investigated the drug resistance of *Acinetobacter bomanii* 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 (6). The results of Rasti et al., Who investigated the *Acinetobacterbomanii* 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%). (7)

In the study of Sadeghifard et al., Who evaluated the resistance level of *Acinetobacter bomanii* strains in Tehran city, the results showed that all isolates of *Acinetobacter bomanii* were resistant to ceftazidime, cefoprazone, ceftazidime, tricarcelin, clavulanic acid, cefotaxime, aztreonam, Meropenem, cefixim, ceftriaxone, carbenicillin, and ticarcylin, but all isolates were sensitive to cholestin (8).

The results of Ahmadnia et al., who investigated antibiotic resistance of *Acinetobacter* 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%) (9).

In the study of Semyon et al., 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% (10). In a study by Boroumand et al.

In Tehran, 53.4% of the samples were resistant to ciprofloxacin and 24.6% resistant to ampicemin (11). In the study by Caroline, 46% of the isolates were resistant to ciprofloxacin and 2% of the samples were resistant to ampicemin (12). The results of zhao study, who

investigated the resistant pattern of *Acinetobacterbomanii* 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 to bramycin (81.5%) (13).

The results of rahbar et al. study on prevalence of antibiotic resistant, showed that resistance to ceftriaxone (90.9%), piperacillin (90.9%), ceftazidime (84.1%), amikacin (2/85%), ciprofloxacin (90.9%) (14).

In the study of Uwingabiye et al., Resistance to antibiotics such as ciprofloxacin, ceftazidime, piperacillin- tazobactam, imipenem, amikacin, to bramycin, dabylmezin, rifampin, colistin were 87%, 86%, 79%, 76%, 52%, 43%, 33%, 32% and 1.7% respectively (15).

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 (16).

In the study of Abhishek et al., The minimum inhibitory concentration of methanol extract against *B.subtilis*, *E.faecalis*, *E.coli*, *K.pneumonia*, *M.Luteus*, *P.vulgaris* and *P. aeruginosa* was equal to 4- 0.01-0.1-2.0mg/ml, Respectively (Abhishek et al., 2013). In Thanawala et al., Inhibition diameter of Acetonic extract of *Tecomellaundulata* was compared to *Baciussubtiluslyse* (17 mm) and *Staphylococcus aureus* (10 mm), while the inhibitory diameter of alcoholic extract of *Tecomellaundulata* against the *Escherichia coli* was 9 mm M (Thanawala et al., 1993).

## **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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