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# Antimicrobial Impact of Hibiscus Sabdariffal Extract Against Acinetobacterbaumannii

**Nagmeh Eskandary[1], Saeide Saeidi[2], Gelareh Sohil Baigi[3], Fereshteh Javadian[4*]**

[1]*Zabol university, Zabol, Iran*
[2]*Institute of Agricultural Biotechnology, University of Zabol, Zabol, Iran*
[3]*Kermanshah University of Medical Sciences, Martyr Chamran Hospital, Kangavar, Iran*
[4]*Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, Iran*

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**ABSTRACT**

**Objective:** Antibiotic resistant to Antimicrobial agent is one of the most important concern in hospitals, which can cause lots of costs, treatment fails and mortality rates. Acinetobacter baumannii is a Gram-negative bacterial pathogen notorious for causing serious nosocomial infections that resist antibiotic therapy. The purpose of current study was to define the evolution of antimicrobial impact of flower extract of *Hibiscus sabdariffal*against*Acinetobacterbaumannii*. **Methods:** Hibiscus tea plant after collecting dried and then milled, their extraction was performed using vacuum from the center (Rotary). The Twelve strains of *Acinetobacterbaumannii* were isolated from patients in the city of Zabol. Minimum inhibitory concentrations and MBC of the extract was determined on bacteria in different concentrations by dilution in the wells. **Results:** The result revealed that the levels of MIC range were from 6.25 to 25ppm. The highest MIC value was 6.25 ppm against *A. Baumannii* and *Acinetobacterbaumannii* were resistance to 4 of the agent including nalidixic acid(100%), penicillin(100%), tetracyclin(58.3%), amikacin(83.3%). **Discussion**: In important human pathogens, drug resistance is increasing according to the results of this study may be proposed that this plant can be used as a drug. It can be a good way to replace herbs with chemical drugs.

## 1.INTRODUCTION

Herbal medicines are the major remedy in traditional medical systems from thousands of years and made a great contribution in maintaining human health and in preventing many infectious diseases(1). The recent reports of the medical community has confirmed that infection with Gram-negative bacteria is increasing because of the resistance of bacteria to antibiotics (2-5). Hibiscus sabdariffa L. (family Malvaceae) is an annual, tropical or subtropical shrub that is native to an area from Malaysia to India. Hibiscus is grown in many countries including Sudan, Mexico, India, and Thailand. While many species of Hibiscus are used as ornamentals, the red calyces of *H.sabdariffa* are used in the preparation of a flavorful and tartcold or hot beverage(6, 7). *H. sabdariffa* is a common herbal drink consumed both hot and cold by people around the world and used in traditional medicine in the treatment of hypertension. The infusion is made from the calyces of the *H. sabdariffa*(8). Relatively few studies have been carried out to evaluate the antimicrobial activities of this plant against common human pathogens (9, 10).

Extracts of the plant significantly enhanced the inhibitory effects of antibacterial agents against different bacterial species (11). The infection caused by *Acinetobacter*is

***Corresponding Author:** Fereshteh Javadian, Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, Iran (Fereshteh.Javadian@yahoo.com)

difficult to control due to multidrug resistance, which limits therapeutic options in critically ill and debilitated patients, specificallythose in intensive care units(12). Hospital outbreaks have been described from various geographic areas and this organism has become endemic in some of them. The role of the environmental contamination in the transmission of nosocomial infections in general and in *A. baumannii* infections in particular is well recognized (13, 14, 15).

### 1.1.Objective

According to the medicinal properties of Hibiscus sabdariffa, there was no comprehensive study in Southeastern Iran; thus, we aimed to study the antibacterial effect of this plant.

## 2.MATERIAL AND METHODS

The different environmental samples were processed for the isolation of bacteria by methods described elsewhere (16).Morphologically distinct colonies obtained from different plates were streaked on Nutrient agar (NA), MacConkeyagar (MCA) and blood agar to purify. The bacterial isolates were identified on the basis of standard cultural, morphological and biochemical characteristics (17).

### 2.1. 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-Iran. *Acinetobacterbaumannii*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 .

### 2.2.Plant materials

The flowers of *Hibiscus sabdariffal* were collected in the region of Iran. The plant materials were dring at 25 °C. Samples were powdered and transferred into glass containers and preserved until the next experiment.

### 2.3. Preparation of extracts:

For each test, 50 grams of samples soaked for 48 hours in 96% ethanol and stored. The extract obtained with smooth filter paper was condensed using rotary. The weight of a test tube determined and one ml of extract was transferred into it. Tube containing extract was dried at room temperature. The weight difference tube was equivalent one ml of the extract. Average three times, was calculated as weight dried extract. Then dissolved in the solvent DMSO and was maintained at 4 °C until used .

### 2.4. Antimicrobial testing of extraction:

Susceptibility of bacterial isolates with multiple resistance to the plant extract of Achillea, green tea and Ajowan was investigated using the dilution plate. To seven micro-titter plates was added amount of the 100 ml nutrient broth Mueller Hinton Broth (MHB). The first well was added 100 ml of the diluted solution of the extract, after mixing, 100 ml of the first plate is removed and added to the second plate, this work was done in this way until the last plate. Remove the end plate 100 ml of culture medium containing $10^7$ units per ml, 100 ml of bacterial suspension equivalent to 0. 5 McFarland added to all wells and incubated at 37 °C for 24 h.

The first well that prevent the growth of bacteria is considered as MIC and to ensure 10 ml of the clear plate was transfered to the Mueller Hinton agar medium. After 24 hours the first dilution that could kill 99.9% of bacteria as shown Minimum lethal concentration.

Agar Well Diffusion Assay for Extracts. Antibacterial activity of the plant crude extracts was tested using agar well diffusion method. The test inoculums (0.5 McFarland turbidity) were spread into Muller-Hinton agar using a sterile cotton swab. The wells were made by sterile well puncture and 20 µL of the extracts were added to each well and incubated at 37°C for 24 hours. The presence of inhibition zone was regarded as the presence of antimicrobial action. The average diameter of the inhibition zone was measured in millimeter.

### 2.4. Statistical Analysis

For all samples, experiments have been repeated three times. Data were imported in Excel then analyses using SPSS. All data were studied with mean representational for bacterial strains and completely random design statistics for fungi samples. A value of $P<0.05$ was regarded as statistically significant.

## 3. RESULT

Antibiotic susceptibility *Acinetobacter baumannii*was evaluated for 4 antimicrobial. However, overall *Acinetobacter baumannii*were resistance to 4 of the agent including nalidixic acid(100%), penicillin(100%), tetracyclin(58.3%), amikacin(83.3%). As was shown in table 1, the most frequent resistance was observed for nalidixic acid and penicillin**.**

**Table 1.** Percentage of antimicrobial susceptibility of 12 strains of *Acinetobacterbaumannii*

| | **NA** | **P** | **AN** | **TE** |
|---|---|---|---|---|
| S | 0 | 0 | 8.3 | 16.6 |
| I | 0 | 0 | 8.3 | 25 |
| R | 100 | 100 | 83.3 | 58.3 |

NA=nalidixic acid, P=penicillin, TE=tetracyclin, AN=amikacin

In this study, the minimum inhibitory concentration (MIC) was used. The levels of MIC were in ranges from 6.25 to 25ppm. The highest MIC value was observedat

6.25 ppm against one. *A. baumannii*(Table 2). The highest MBC for *Hibiscus sabdariffal* leave was 50 ppm, while the lowest MBC was 12.5 ppm. The maximum diameter of inhibition zone (mm 14) for *Hibiscus sabdariffal* leaves was observed against *A. Baumannii*.

**Table2:** Minimum inhibitory concentration of Hibiscus sabdariffal extract against A. Baumannii

| Bacterial cod | MIC and MBC(PPM) | Bacterial cod | MIC and MBC(PPM) |
|---|---|---|---|
| 1 | 12.5/ 25 | 7 | 6.25 / 12.5 |
| 2 | 25/ 50 | 8 | 12.5/ 25 |
| 3 | 25/ 50 | 9 | 25/ 50 |
| 4 | 12.5/ 25 | 10 | 12.5/ 25 |
| 5 | 12.5/ 12.5 | 11 | 12.5/ 25 |
| 6 | 12.5/ 12.5 | 12 | 12.5/ 12.5 |

**Table3:** Antimicrobial Activity of the Plant Crude Extracts as. Mean of Inhibition Diameter Zone Against A. Baumannii

| Bacterial cod | Diameter Zone for Leaf Extract, mm | Bacterial cod | Diameter Zone for Leaf Extract, mm |
|---|---|---|---|
| 1 | 10 | 7 | 14 |
| 2 | 5 | 8 | 9.5 |
| 3 | 4 | 9 | 4 |
| 4 | 9 | 10 | 10 |
| 5 | 10 | 11 | 11.5 |
| 6 | 11 | 12 | 10 |

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**Authors' Contributions**
All the authors had equal roles in design and writing of the manuscript.

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