



## Antibacterial and Antibiofilm Effects of Ethanol and Aceton Leaf Extract of *Momordica charantia* and *Tecomella undulata* against *Acinetobacter baumannii*

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### ABSTRACT

**Background:** This study considered the increasing resistance of bacteria to antibiotics and the presence of antibacterial agents in plants.

**Objectives:** The aim of this study was studying the antimicrobial and antibiofilm activity of *Tecomella undulata* and *Momordica charantia* ethanol and aceton leaf extract on antibiotic resistance *Acinetobacter baumannii*.

**Methods:** The leaves of *Tecomella undulata* and *Momordica charantia* were collected from Saravan city and extracted by rotary machine. *Acinetobacter baumannii* 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 lowest MIC and MBC of ethanol leaf extract of *Tecomella undulata* was 0.62 and 1.25mg/mL, respectively. The lowest MIC and MBC of aceton leaf extract of *Tecomella undulata* was 0.31 and 0.62mg/mL, respectively. The lowest MIC and MBC of ethanol leaf extract of *Momordica charantia* was 1.25 and 2.5 mg/mL, respectively. The lowest MIC and MBC of aceton leaf extract of *Momordica charantia* was 0.31 and 0.62 mg/mL, respectively. The resistance of the strains was to amoxiclavanic (10%), ampicillin (20%), gentamicin (0%), ceftazidime (0%) and nitromicin (0%) antibiotics. The aceton extract had more effect on *Acinetobacter baumannii* than ethanolic extract. The effect of *Momordica charantia* and *Tecomella undulata* against *Acinetobacter baumannii* were the same.

**Conclusion:** Considering the results obtained and increasing resistance of bacteria to chemical antibiotics, it is suggested that bacterial compositions of these plants can be used to treat bacteria.

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**Key words:** *Acinetobacter baumannii*; Antimicrobial Activity; Biofilm effects; *Momordica charantia*; *Tecomella undulata*

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## Introduction

*Acinetobacter baumannii* is a gram-negative, cocco-basil-free, immobile bacterium. *Acinetobacter baumannii* causes rarely severe infections in people with normal levels of immunity, and is also less commonly known as the natural flora of a healthy person. Different strains of *Acinetobacter baumannii* are often resistant to antimicrobial drugs, which can be inherent or through the acquisition of genetic factors. Studies show that clinical *Acinetobacter baumannii* isolated from Iranian hospitals are highly resistant to several classes of antibiotics (Karimipour et al., 2016).

The prevalence of *Acinetobacter baumannii* has increased in most hospitals due to the increasing variety of surgical procedures, the use of a variety of antibiotics, and the presence of hosts with immunodeficiency. The very presence of unique genetic capabilities in this organism has led to the production of factors involved in their resistance to harsh environments, as well as the creation of widespread epidemics and its spread around the world due to the antibiotic resistance of multidrugs, has led to this issue. The organism has attracted the attention of many researchers over the past two decades (Mahmudpour et al., 2019).

Multiple drug-resistant *Acinetobacter baumannii* depends on a variety of factors, such as environmental pollution and contact with health care providers who have colonization of the organism. Non-addictive factors that lead to some species of *Acinetobacter baumannii* also include hospitalization in beds with a bed size above 500, recent antibiotic treatments, surgery on the urinary system, genitals, and previous hospitalization in the intensive care unit (Mahmudpour et al., 2019).

Because few antibiotics are effective against these organisms, and because of the increased risk of antibiotic resistance, it is important to find new antimicrobial compounds with high lethality and the least potential for microbial resistance. Using herbal medicine to fight bacterial infections is one of the most effective methods.

Lapachol is a naphthoquinone with anticancer, antibacterial, antifungal and antiviral activities. Lapachol and some of its derivatives tested with good experimental results in tumors. The bark of some trees belonging to the Bignoniaceae family contain up to a few percent of lapachol and often a considerable amount of related compounds. *Tecomella undulata* (Roxb.) Seem is the only native species of the family in arid and semi-arid of southern parts of Iran. *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 (Mohsenzadeh et al., 2010).

In addition, its distribution in Afghanistan, West Pakistan and southeastern Arabia has been recorded (Mozaffari and Abbasi, 2005). Due to medicinal properties, this plant has been considered as a good treatment (Jain et al., 2012). Flavonoid compounds, Phytosterol, flavonol, fatty acids, and terpenes have been identified in various parts of the plant (Laghari et al., 2013). It has anti-inflammatory, antimicrobial and anti-oxidant activity (Khatri et al., 2009; Laghari et al., 2013). 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 (Fazeli-Nasab, 2018; Khare, 2008).

*Momordica charantia* (Family *Cucurbitaceae*) are used in the Amazon, Brazil and parts of Asia, among its many uses, for treatment of skin infections. But also the plant is grown in some parts of East Africa, Tanzania inclusive, where it is locally known as *Zukini* and used as appetizer among

other utilities. The fruits and leaves contain alkaloids, glycoside, saponin-like substances, resin, an aromatic volatile oil and mucilage. Reports also show that the plant has anti-tumor and anti-HIV activities (Grover and Yadav, 2004; Nagasawa *et al.*, 2002). A leaf tea is used for diabetes, to expel intestinal gas, promote menstruation, and as anti-viral agent against measles and hepatitis viruses (Ahmed *et al.*, 2001).

Billions of years of selective pressures have given rise to numerous strategies in bacteria survival, which adapts this organism to almost any environmental niches. One of preferred growth states for bacteria is known as biofilm which exists in more than 90% of bacteria. Biofilms are multicellular surface-attached communities of bacteria embedded in extracellular matrix (ECM). Quorum sensing (QS), a cell-to-cell communication, has been identified to play critical roles in formation of biofilm with its surrounding ECM. Bacteria living in biofilms show a highly elevated pattern of adaptive resistance to antibiotics and other disinfectants compared to their planktonic compartments. Increasing Adaptive antibiotic resistance globally acts as an obstacle when treating biofilm-associated acute and chronic infections (Costerton *et al.*, 1999), such as nosocomial pneumonia cases, surgical wound infections, catheter-associated infections, burn wound infections, ventilator-associated pneumonia, etc. Biofilm-forming has thus caused a large number of problems in health care, food industry, and other fields (Jakobsen *et al.*, 2017).

The increase in diseases caused by the consumption of food contaminated with pathogenic or toxin-laden bacteria or their toxins has for many years been considered as an important and vital issue for the general health and well-being of foodborne pathogens. Outbreaks appear to be exacerbated during pregnancy (Mashreghi and Momtazi, 2012) and also based on this idea that medicinal plants have many therapeutic properties including antimicrobial effects with minimum side effects as the result, the purpose of this study was to evaluate the Antibacterial and antibiofilm activity of the ethanol and acetone extract of *Tecomella undulata* and *Momordica charantia* on the antibiotic-resistant *Acinetobacter baumannii* in Zabol.

## Materials and methods

### Material

In this study 20 isolates of *Acinetobacter baumannii* (Figure 1) from infected patients (the patients age was between 23 to 64) in Imam Khomeini and Ali ibn Abi Talib hospitals in Zabol were investigated.



**Figure 1.** the leaf and flower of *Tecomella undulata*

### Laboratory procedures

The clinical specimens were cultured on the McCanky Agar and Blood Agar medium ((Cat. no. Q63; Santa Maria, CA; USA)(5.2 g CM0007 powder, 0.4 g CaCl<sub>2</sub>, 75 mL distilled water) overlaid with a pectate-EDTA layer (0.1% EDTA containing 2% sodium polypectate)) are inoculated at 37 °C for 24-24 h (Gregersen, 1978).

Samples were isolated on McCanky agar medium and incubated again at 37 °C. Colonies were examined for microscopic (gram staining) and macroscopic (colony morphology) characteristics. Oxidase tests were performed for suspected colonies. In the negative, they were inoculated in of medium and then incubated at 37 °C. In case of oxidation, the bacteria were cultured in Müller Hinton agar medium and incubated at 42 °C (Bottone and Hanna, 1978; Gregersen, 1978; Sivendra *et al.*, 1975).

An oxidase test was performed in case of growth after gram staining and observation of cocci and gram negative diplococci. In the next step, by using biochemical tests, cultured on McCanky agar and incubated at 37 °C and 42 °C, then citrate and moving test were performed on the OF media containing glucose (Johnson *et al.*, 1995).

### Determination of antibiotic susceptibility

Determination of susceptibility was done by standard Disc diffusion agar. For testing, bacterial colonies, 0.5 µM MacFarland suspension was 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(Anand *et al.*, 2009; Bauer, 1966; Nicodemo *et al.*, 2004).

### Preparation of ethanolic leaf extract

*Tecomella undulata* and *Momordica charantia* leaves were collected from Saravan city and dried. To prepare the ethanolic leaf extract, 10 grams of dried powder were placed inside half-liter erlenn containing 100 mL of ethanol (96 %)(to prepare the ethanolic leaf extract). The contents of the erlenn were mixed at room temperature for 24 hours by shaker machine (Iran) at 130 rpm, then filtered with Wattman No. 2 paper. Solvent separation from the leaf extract was performed by a

rotary machine (Heidolph-Germany) with the aid of a vacuum pump (vacuum distillation). The leaf extracts were weighed and then solved in DMSO solvent (CR8014 model; scharlau; Brazil). The leaf extract was stored in a refrigerator until use in Antibacterial experiments at 4 °C (Ashraf *et al.*, 2016; Lee and Kim, 2014).

### **Determination of susceptibility of bacterial strains to different leaf extracts**

Determination of susceptibility of bacterial strains to plant leaf 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 µL of diluted solution of the leaf 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/m which was equal to 0.5 McFarland added and incubated at 37 °C for 24 hours. The first pill that was prevented bacterial growth after placing in the incubator was considered as the minimum inhibitory concentration (MIC). In order to ensure, 10 µL 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 (Jahan *et al.*, 2011).

### **Biofilm formation assay in the presence of the biocides**

After performing the procedure described above, the microplate was covered and incubated aerobically for 24 h at suitable temperature. At first, the OD (Optical Density) was measured (600 nm) by using an automated ELISA counter, then, the content of each well of the microplate was aspirated and each well was washed three times with 250 µL of sterile physiological saline. The remaining attached bacteria were fixed with 200 µL of 99% ethanol per well and after 15 min all of the wells were discarded and left to dry. Then, each well was stained for 5 minute with 0.2 mL of 2% crystal violet. Excess stain was rinsed off by washing the plate slowly with distilled water. After the plate was air dried, the dye bound to the adherent cells was solubilized with 160 mL of 33% (v/v) glacial acetic acid per well. The OD of each well was measured at 492 nm by using an automated ELISA counter (Houari and Di Martino, 2007; Srinivasan *et al.*, 1995).

### **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).

The results of this study showed that the lowest inhibitory concentration of ethanol *Tecomella undulata* was 0.31 mg/mL, of which 6 strains were inhibited at this concentration, while the highest inhibitory concentration was 5 mg/mL. Three strains have been inhibited in this concentration. The highest bactericidal concentration was 10 mg/mL, in which 2 strains were eliminated at this concentration, while the lowest bactericidal concentration was 1.25 mg/mL (Table 2).

The results also indicated that the MIC of acetone extract against *Acinetobacter baumannii* was 0.31 mg/mL with two strains inhibited at this concentration. The highest inhibitory concentration of acetone extract was 5 mg/mL with five strains inhibited at this concentration (Table 2).

Further, the results demonstrated that the biofilm formation decreased with increasing concentration of the extract so that the acetone extract had more inhibitory effect on biofilm formation.

It was additionally shown that the lowest inhibitory concentration of *Momordica charantia* ethanol extract was 1.25 mg/mL, in which 4 strains were inhibited, while the highest inhibitory concentration was 10 mg/mL with 3 strains inhibited in this concentration. The lowest inhibitory concentration of acetone extract of *Momordica charantia* was 0.31 mg/mL, in which 6 strains were inhibited and 2 strains were eliminated in all concentrations of acetone extract.

The lowest lethal concentration of ethanolic of *Momordica charantia* extract was 2.5 mg/mL, with 4 strains inhibited at this concentration, and the highest lethal concentration was 20 mg/mL (Table 3). The results of microfilm biofilm testing showed that in higher concentrations of the extract, the rate of biofilm formation decreased.

The acetone extract had more effect on *Acinetobacter baumannii* than ethanolic extract. The effect of *Momordica charantia* and *Tecomella undulata* against *Acinetobacter baumannii* were the same.

**Table 1.** Antibiotic pattern against strain bacteria

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

**Table 2.** MIC and MBC of ethanol and acetone leaf extract of *Tecomella undulata* against Strain bacteria

MIC/MBC of acetone extract	MIC/MBC of Ethanolic extract	Strain bacteria
1.25-2.5	0.31-0.62	1
0.62-1.25	2.5-5	2
5-10	0.62-1.25	3
1.25	0.31-0.62	4
2.5	1.25-2.5	5
5-10	0.31-0.62	6
5-5	2.5-5	7
6.25-1.25	0.62-1.25	8
2.5-5	2.5-5	9
0.31-0.62	0.31-0.62	10
5-10	1.25-2.5	11
0.62-0.62	0.62-1.25	12
1.25-2.5	0.62-1.25	13
1.25-2.5	1.25-2.5	14
0.31-0.62	2.5-5	15
2.5-5	5-10	16
2.5-5	0.31-0.62	17
0.62-1.25	1.25-2.5	18
1.25-2.5	1.25-2.5	19
5-10	0.31-0.62	20

**Table 3:** MIC and MBC of ethanol and acetone leaf extract of *Momordica charantia* against Strain bacteria

Strain bacteria	MIC/MBC of Etanol extract	MIC/MBC of Aceton extract
1	10-20	0.62-1.25
2	2.5-5	1.25-2.5
3	2.5-5	0.31-0.62
4	1.25-2.5	0.31-0.62

5	1.25-2.5	-
6	5-10	0.31-0.62
7	2.5-5	-
8	2.5-5	0.62-1.25
9	5-10	1.25-2.5
10	1.25-2.5	0.31-0.62
11	2.5-5	2.5-2.5
12	5-10	1.25-1.25
13	5-10	1.25-1.25
14	2.5-2.5	2.5-5
15	10-20	1.25-2.5
16	1.25-2.5	2.5-5
17	5-5	0.31-0.62
18	2.5-5	0.62-1.25
19	10-10	0.62-1.25
20	2.5-5	0.31-0.62

## Discussion

Although most sequenced strains of *A. baumannii* carry a *bap* gene, many seem to have disrupted or truncated *bap* sequences, which might be attributed to recombination events or sequence alignment errors that are common to the highly repetitive elements of *bap* coding sequences. *bap* has been shown to express a highly variable protein. Partial sequencing of *bap* performed on the index case strain MS1968, revealed that *bap* is a large and highly repetitive gene of approximately 16 kb in size (Goh *et al.*, 2013).

The prevalence, expression, and function of Bap in 24 carbapenem-resistant *A. baumannii* ST92 strains isolated from a single institution were previously investigated (Goh *et al.*, 2013). Out of the 24 strains, 22 expressed Bap. The biofilm-forming ability of 65 MDR *A. baumannii* isolates and its association with biofilm-related genes was assessed using RT-qPCR. *ompA* and *csuE* genes were detected in all isolates; however, *bap* and *blaPER-1* were detected in 43 (66%) and 42 (64%) of the phenotypes that showed strong and moderate biofilm activities ( $P \leq 0.05$ ), respectively (Loehfelm *et al.*, 2008). It was found that the frequency of genes related to biofilm formation in 27 *A. baumannii* isolates were *ompA* (100%), *bap* (30%), and *blaPER-1* (44%) (Badmasti *et al.*, 2015). Western blotting revealed that all the isolates expressed OmpA and only eight isolates were positive for Bap factor (Badmasti *et al.*, 2015).

The study of Kazemi Pour (Kazemi-Pour *et al.*, 2011) Plasmid-mediated antibiotic resistance was observed in selected isolates of *A. baumannii* and experiments of conjugation and transformation showed the occurrence of gene transfer. Plasmid curing was used to examine the function of plasmids. Five plasmids of *A. baumannii* A3 were cured but no differences were observed between wild-type and plasmid-cured strains with respect to the biofilm formation capabilities.

The study of Bala, on testing by tissue culture plate method, 12 (16%) *Acinetobacter* isolates were weak biofilm producers, 9 (12%) were moderate producers, 30 (40%) were strong biofilm producers, 24 (32%) isolates were nonbiofilm producers and 63 % isolates were multidrug resistant (Bala *et al.*, 2016).

It is reported that that AgNPs have strong anti bacterial and anti biofilm activity against the examined pathogens. Synergistic effects of AgNPs in combination with gentamicin, kanamycine,

cephalosporin and penicillin were observed in different cases. *Ps. aeruginosa* and *S. aureus* showed more sensitivity (increase in fold) to examined antibiotics plus AgNPs compared to *A. baumannii* and *E. faecalis*. Our results demonstrated that AgNPs showing promising anti biofilm activity on hospital isolates of *Ps. aeruginosa*, *A. baumannii*, *E. faecalis* and *S. aureus*. The study also demonstrated a possible combination of examined antibiotics with AgNPs which concluded as synergism (Ebrahimi *et al.*, 2018)

The results of this study showed that the strains were resistant to amoxiclalanic antibiotics (10%), ampicillin (20%), gentamicin (0%), ceftazidime (0%) and nitromicin (0%). Of course, this result was reported in previous research that it is related that the lowest inhibitory concentration of *Tecomella undulata* is 0.31 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 (Saravani and Javadian, 2020).

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 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 (Angoti *et al.*, 2014).

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%) (Mahmoudi *et al.*, 2016; Vahedi *et al.*, 2018).

In the study of Sadeghifard *et al.*, 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, tricarcetin, clavulanic acid, cefotaxime, aztreonam, Meropenem, cefixim, ceftriaxone, carbenicillin, and ticarcylin, but all isolates were sensitive to cholestin (Sadeghi Fard *et al.*, 200; Soroush *et al.*, 2010).

The results of Ahmadikiya *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%) (Ahmadikiya *et al.*, 2017).

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% (Simhon *et al.*, 2001). In a study by Boroumand *et al.* In Tehran, 53.4% of the samples were resistant to ciprofloxacin and 24.6% resistant to ampicemin (Boroumand *et al.*, 2009).

Caroline's inquiry revealed that 46% of the isolates were resistant to ciprofloxacin and 2% of the samples were resistant to ampicemin (Henwood *et al.*, 2002).

Zhao investigated the resistant pattern of *Ainetobacter baumannii* and reported 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%) (Zhao *et al.*, 2015).

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%) (Rahbar *et al.*, 2010).



Uwingabiye et al. delved into resistance to antibiotics such as ciprofloxacin, ceftazidime, piperacillin-tazobactam, imipenem, amikacin, tobramycin, dabylmezil, rifampin, colistin which were reported to be 87%, 86%, 79%, 76%, 52%, 43%, 33%, 32% and 1.7%, respectively (Uwingabiye et al., 2016).

In recent decades, the research priority has been drawn 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 (Fazeli-Nasab et al., 2018; Overbye and Barrett, 2005).

Over the past decade, we have witnessed the presence of *Acinetobacter baumannii* as the most successful hospital pathogen worldwide. Part of this report was about the ability of this microorganism to survive in hospital settings and to acquire the mechanism of resistance and cause acute infections, especially in critically ill patients. Although much information is available on the mechanisms responsible for antibiotic resistance in this microorganism, we are still unable to stop it. Today we see strains of universal resistance and even resistance to colistin that have severely restricted treatment (Peleg et al., 2008).

Extracts of leaf and stem of *Tecomella* are also reported to be effective against *Salmonella typhi*, the causal organism of typhoid fever (Gehlot and Bohra, 2000).

Antibacterial activity of hexane, chloroform and methanol extracts of *T. undulata* leaves were checked on bacterial pathogens of humans using agar well diffusion method. The methanol extract showed higher antibacterial activity with higher MIC against *Klebsiella pneumoniae* and *Micrococcus luteus*; whereas, less inhibitory effect was noted for chloroform and hexane extracts. Gram-negative bacteria were more susceptible than gram-positive (Abhishek et al., 2013).

*A. marina* leaf extract had a significant antibacterial effect on all 6 strains at a concentration of 100 µL in agar well diffusion method. MICs were 25 µg/mL in all strains. MBCs were 12.5 µg/mL in standard strains. The most resistant strain against the extract was cefepime-resistant clinical strain. *A. marina* leaf crude extract had significant antibacterial effects on standard and carbapenem-resistant clinical isolates of *A. baumannii* (Mahmudpour et al., 2019).

In the study by Abhishek et al., the MIC of methanol extract against *B. subtilis*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *M. luteus*, *P. vulgaris* and *P. aeruginosa* was equal to 4-0.01-0.1-2.0 mg/mL, respectively (Abhishek et al., 2013). In Thanawala et al.'s investigation, 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 (Thanawala and Jolly, 1993).

In any research was studied the Antibacterial activity against five important strains viz. *S. epidermidis*, *B. subtilis*, *P. pseudoalcaligenes*, *P. vulgaris*, and *S. typhimurium*. They showed the inhibition of *S. epidermidis* and *B. subtilis* by methanolic extract of *Tecomella* (Parekh et al., 2006). Research has disclosed that the effect of *Satureja khuzestanica* alcoholic essence on the expression of *Acinetobacter baumannii* biofilm gene could be used as a treatment or complementary therapeutic in infections caused by this bacterium and virulence gene inhibition. The analysis of alcoholic essential oil of *Satureja khuzestanica* showed that the essence has 88.90% carvacrol and 0.3mG/mL MIC (Bahador et al., 2015).

The evaluation of the antimicrobial activity of *Momordica charantia* extracts on reference strains and microorganisms isolated from clinical specimens revealed that antimicrobial activity was observed against all the tested microorganisms with exception to *P. mirabilis* and *C. neoformans*. Methanolic crude extracts exhibited relatively broader antimicrobial spectrum of activity than petroleum ether extracts with the as lower concentration as 0.075 mg/μl. Methanolic fruit crude extract displayed the broadest antimicrobial spectrum by inhibiting majority (75%) of the tested microorganisms. Neither was there synergistic nor addition effect upon mixing leaf and fruit extracts of equal concentrations derived from the same solvent (Mwambete, 2009).

It is related that if *Momordica charantia*, better known as bitter melon, had any antibacterial property as claimed by many food and nutrition specialists. The result show that after 24 hours of incubation, petri dishes containing *S. aureus* with Penicillin and Erythromycin disks showed clear zones of inhibition (average 12.9 mm, and 9 mm respectively). This was similar to the Gentamicin disks for *E. coli* (average 11 mm). There were no clear zones of inhibition around the unmedicated disks or the ones soaked in distilled water or bitter melon extract (interior, middle or exterior skin). Bitter melon extract did not change the color of liquid microKwik media containing the bacteria (Ghosh, 2014). The chloroform fraction also presented the lowest MIC against the same microorganism (64 μg/mL). The ethyl acetate fraction presented a similar result, showing activity against *E. coli* (27) and *B. cereus* (ATCC 33018) at 64 μg/mL (Costa et al., 2010).

*In vitro* antimicrobial activity of leaf extract was seen against *Escherichia coli*, *Salmonella paratyphi*, *Shigella dysenteriae*, and *Streptomyces griseus* in a rearch study that examined the antibacterial activities of *Carum copticum* extracts on the development of microbial biofilms and planktonic form of six pathogenic bacteria (Omogegbe et al., 1996). According to disc diffusion test (MIC and MBC) the ability of *C. copticum* extracts for inhibition of bacteria in planktonic form was confirmed. The best inhibitory effect of this plant on *S. aureus* and low inhibitory effect on *A. baumannii* in planktonic forms were observed. These extracts were efficient to inhibit biofilm structures and concentration of each extract had direct relation with inhibitory effect. The maximum and minimum inhibitory effects of *C. copticum* methanolic extract on biofilm formation were observed on *A. baumannii* (98%) and *K. pneumoniae* (19%), respectively (Mohammadi et al., 2019).

The acetone extract had more effect on *Acinetobacter baumannii* than ethanolic extract. This results are the same as that of previous research (Fazeli-nasab et al., 2019).

## Conclusions

Considering the obtained results and the increasing resistance of bacteria to chemical antibiotics, it is suggested that more studies on antibacterial compounds of these plants in treatment of bacterial infections be conducted. The acetone extract had more effect on *Acinetobacter baumannii* than ethanolic extract. The effect of *Momordica charantia* and *Tecomella undulata* against *Acinetobacter baumannii* were the same.

This research was done *in vitro* and largely prevented biofilm formation. One of the reasons for the inhibition is related to the active ingredients of the plants which largely prevent the bacterial attachment to the surfaces of biofilm formation. *In vivo* studies have not been performed in this study and further research is needed to understand the mechanism of action of these materials and host epithelial cells.

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