



## Study the Antimicrobial Effects of *Momordica charantia* on Pathogenic Bacteria

Khadijeh Saravani<sup>1</sup>, Elham Javadian<sup>2</sup>, Mahboubeh Mohsenbeygi<sup>3</sup>,  
Mehdi Hassanshahian<sup>3\*</sup>

<sup>1</sup> Department of Internal Medicine, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran.

<sup>2</sup> Zahedan University of Medical Sciences, Zahedan, Iran.

<sup>3</sup> Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.

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### ABSTRACT

**Background:** The aim of this study was to investigate the antimicrobial effects of *Momordica charantia* against pathogenic bacteria.

**Methods:** Extract of *M. charantia* species was extracted using Rotary device. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of extract on mentioned bacteria were determined using micro dilution broth method at six different concentrations.

**Results:** The results of this study showed that the lowest inhibitory concentration of extract against bacteria was 12.5 ppm, (*Vibrio cholera*, *Pseudomonas aeruginosa* and *E. coli*) the results also showed that only one inhibited. However, the highest inhibitory concentration was estimated 25 ppm against *Shigella dysenteriae* and *Bacillus cereus* and highest bactericidal concentration was estimated 25 and 50 ppm.

**Conclusion:** The results of this study show good antimicrobial effects of *M. charantia* on pathogenic bacteria and these medicinal plants can be used to treat infections caused by bacteria.

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\*Corresponding Authors: Mehdi Hassanshahian, Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.

Tel: +98-34-329641, E-mail: mshahi@uk.ac.ir

## Introduction

*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 (1, 2, 3). A leaf tea is used for diabetes, to expel intestinal gas, promote menstruation, and as anti-viral agent against measles and hepatitis viruses. Antioxidant, anti-diabetes, anti-inflammatory, anti-bacterial and anti-cancer effects of *M. charantia* have been reported (3, 4).

Fruits and seeds of *M. charantia* possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial and antitumor (2). The plant was generally used to investigate for immune-stimulant activity, chemo-taxis stimulation, treating ulcers, anti-hyperglycemic and hypoglycemic activity and antioxidant enzyme activities in Turkey (5, 6, 7).

*Pseudomonas aeruginosa* is responsible for 10–15 % of the nosocomial infections worldwide (8). Often these infections are hard to treat due to the natural resistance of the species, as well as to its remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents. *P. aeruginosa* represents a phenomenon of antibiotic resistance, and demonstrates practically all known enzyme and mutational mechanisms of bacterial resistance (9). *Bacillus cereus* is a spore forming bacterium that produces toxins that cause vomiting or diarrhea. Symptoms are generally mild and short-lived (up to 24 hours). *B. cereus* is commonly found in the environment (e.g. soil) as well as a variety of foods. Spores are able to survive harsh

environments including normal cooking temperatures.

*Shigella* is a Gram-negative, non-motile bacillus belonging to the Enterobacteriaceae family. There are four species of *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* (designated as serogroups A, B, C and D respectively). The first three species include several 19 serotypes. Acquired immunity to *Shigella* is serotype - specific. While *S. boydii* and *S. sonnei* usually cause a relatively mild illness (watery or bloody diarrhea only), *S. flexneri* and *S. dysenteriae* are chiefly responsible for endemic and epidemic shigellosis, respectively, in developing countries, with high transmission rates and significant case fatality rates.

*Escherichia coli* is a Gram negative rod (bacillus) in the family Enterobacteriaceae. Most *E. coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins. Pathogenic *E. coli* can be classified into pathotypes by their virulence factors, together with the type of disease.

## Materials and Methods

### *Bacterial strains and culture conditions*

Bacterial strains were obtained from standard laboratory of veterinary department Zabol University, Zabol, Iran. To evaluate the antibacterial activity the plant extracts were investigated using strain of bacteria *Pseudomonas aeruginosa* ATCC27853, *Bacillus cereus* PTCC1015, *Shigella dysenteriae* PTCC 1188, *E. coli* ATCC25922, *Vibrio cholera* ATCC1611. The typed cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4°C until required for study.

### Plant materials

The leaves of *M. charantia* were collected in Zabol and plants were deposited in herbarium of Zabol University. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

### Preparation of extracts

Plants were properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 40C in air tight screw-cap tube.

### Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Susceptibility of bacterial isolates to the plant extracts was determined using the serial dilution method.

### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

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 6.25 ppm to 100 ppm. To each well, 10  $\mu$ L of indicator solution (prepared by dissolving a 10 mg extract in 2 mL of DMSO) and 10  $\mu$ L of Mueller Hinton Broth were added. Finally, 10  $\mu$ L of bacterial suspension (106 CFU/mL) was added to each well to achieve a concentration of 104 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated.

The plated were prepared in triplicates, and then they were placed in an incubator at 37 °C for 18 - 24 hours. 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 and MBC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganisms does not demonstrate the visible growth. The microorganism's growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganisms was completely killed.

### Result

The results of this study showed that the lowest inhibitory concentration of extract against bacteria was 12.5 ppm, (*Vibrio cholera*, *Pseudomonas aeruginosa* and *E. coli*). Also, among the studied bacteria only one bacterium was inhibited. However, the highest inhibitory concentration was estimated 25 ppm against *Shigella dysenteriae* and *Bacillus cereus* and highest bactericidal concentration was estimated 25 and 50 ppm (Table 1). The results also showed that the highest inhibitory hole diameter at 50 ppm was  $24 \pm 1$ , which was observed against *Escherichia coli* bacterium while the lowest inhibitory hole diameter was observed against *Bacillus* isolate (Table 2).

**Table 1.** MIC and MBC *Momordica charantia* extract against pathogen bacteria.

	MIC	MBC
<i>Shigella dysenteriae</i>	25	50
<i>Bacillus cereus</i>	25	50
<i>Vibrio cholera</i>	12.5	25
<i>Pseudomonas aeruginosa</i>	12.5	25
<i>Escherichia coli</i>	12.5	25

**Table 2.** Antimicrobial screening test of ethanolic plants extract against some bacterial strains.

Bacteria	Concentration (ppm)			
	6.25	12.5	25	50
<i>Shigella dysenteriae</i>	4±1	9±1	15±1	17±1
<i>Bacillus cereus</i>	3±1	6±1	12±1	16±1
<i>Vibrio cholera</i>	10±1	16±1	18±1	20±1
<i>Pseudomonas aeruginosa</i>	14±1	18±1	20±1	21±1
<i>E. coli</i>	16±1	20±1	22±1	24±1

## Discussion

According to Omoregbe et al. (1996) aqueous, ethanolic and methanolic extracts of *M. charantia* leaves presented antimicrobial activity against *E. coli*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Streptomyces griseus* and *Mycobacterium tuberculosis* (10, 11, 12, 13, 14). On the other hand, Prabakar and Jebanesan (2004) have shown that the leaves methanolic extract (11, 15, 16, 17) has been effective against *Culex quinquefasciatus* larva. The study of Mada, the diameter of zones of inhibition obtained ranged from 17 to 14 and 15 to 11 mm for ethanol and aqueous extracts, respectively. The minimum inhibitory concentration (MIC) values ranged from 6.5 to 12.5 mg/ml for the ethanol extract and 12.5 to 50 mg/ml for the aqueous extract. Similarly, the minimum bactericidal concentration MBC values ranged from 12.5 to 25 mg/ml for the ethanol extract and 50 To 100 mg/ml for the aqueous extract (12, 18, 19, 20, 21).

The broad spectrum anti-microbial activity obtained from the aqueous and ethanol leaves extract of *M. charantia* was in agreement with the work of Jagessar et al.(2008)(13). Ankita et al. (2012) also reported broad spectrum antibacterial activity against some pathogenic bacteria by *M. charantia* (Cucumber) and *Praecitrullus fistulosus* (Tinda) (14, 22, 23, 24, 25).

The study of Mwambete, 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 (15, 26, 27, 28).

The study of Leelaprakash was to investigate the in vitro antimicrobial and antioxidant activity of aqueous and methanol extracts of *Momordica charantia* leaves. Methanolic plant extract showed a maximum zone of inhibition in *E. coli* by disc method, but in well diffusion method *Bacillus* and *Klebsiella* showed maximum inhibitory activity (16, 29, 30, 31).

The study of Braca, The essential oil obtained from the seeds of *M. charantia* was analyzed by GC/MS. Twenty-five components, representing 90.9% of the oil, were identified. The oil was tested for its antibacterial and antifungal activities. *Staphylococcus aureus* was found to be the most sensitive microorganism with MIC values < 500 μg/ml (17, 32, 33).

## Conclusion

The results of this study showed that *M. charantia* has good antimicrobial effects against Pathogen bacteria. Although the clinical application of herbal extracts and essential oils due to their lower side effects and their lower cost of production is beneficial and cost effective, but it seems that for clinical application of *M. charantia* extracts, more studies and researches should be undertaken on the mechanism of effective compounds action and also further studies should be conducted on microbial agents, pharmacological activity and pharmacokinetics of this plant.

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## Ethics approval and consent to participate

This project was approved by the Ethics Committee of Zabol University and code project IRUOZ.ECRA. 2019.001.

## Conflict of interest

The authors declare no conflict of interest.

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