



ARTICLE

Antibacterial Activity of *Momordica charantia* Seed Extracts Against Selected Gram-Positive and Gram-Negative Bacterial Pathogens

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Abstract

Momordica charantia (bitter gourd) has long been used in traditional medicine and possesses multiple bioactive phytochemicals with potential antimicrobial properties. This study was aimed to evaluate the antibacterial activity of *Momordica charantia* seed extracts against selected Gram-positive and Gram-negative bacterial pathogens using the Kirby–Bauer disc diffusion method. Seed extracts of *M. charantia* were prepared and tested against Gram-positive bacteria and Gram-negative bacteria at a concentration range of 100–800 mg/mL. The strongest inhibition among Gram-positive bacteria was observed against *Listeria monocytogenes* at all concentrations and

highest at 700 mg/mL, whereas for *Staphylococcus aureus*, inhibition was between 600–700 mg/mL with highest at 700 mg/mL. However, for *Bacillus cereus* the inhibition was observed between 500–800 mg/mL. As for Gram negative bacteria, inhibitory activity was observed against *Acinetobacter baumannii* from 700–800 mg/mL and *Enterobacter cloacae* at 800 mg/mL. No significant antibacterial activity was observed against *Streptococcus mutans*, *Klebsiella pneumoniae*, or *Salmonella enteritidis*. *Momordica charantia* seed extract possesses selective antibacterial activity against several clinically relevant microorganisms, particularly Gram-positive bacteria and certain multidrug-resistant Gram-negative pathogens. These findings support the medicinal value of *M. charantia* seeds and highlight its potential as a source of novel antibacterial compound.

Citation

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1 Introduction

Medicinal plants continue to play a major role in Infectious diseases, particularly in developing countries where access to conventional medicines may be limited [1, 2]. In recent decades, there has been increasing scientific interest in evaluating medicinal plants for pharmacological activities and antimicrobial properties.

The emergence of multidrug-resistant microorganisms has become a major global health challenge [1]. Resistance among clinically important bacteria such as *Staphylococcus aureus*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* has significantly reduced the effectiveness of currently available antibiotics. Consequently, there is an urgent need to

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identify alternative antimicrobial agents from natural sources [3, 4].

Momordica charantia L., commonly known as bitter melon or bitter melon, belongs to the Cucurbitaceae family and is widely cultivated throughout Asia, Africa, and tropical regions. Traditionally, the plant has been used for the treatment of diabetes mellitus, gastrointestinal disorders, infections, inflammation, and various other conditions [5, 6]. Previous studies have demonstrated that *M. charantia* contains numerous bioactive compounds including alkaloids, flavonoids, saponins, triterpenoids, glycosides, and charantin, many of which possess antimicrobial and antioxidant properties [7, 8].

Although several studies have investigated the medicinal properties of *M. charantia*, limited data are available regarding the antibacterial activity of its seed extracts against a broad spectrum of clinically significant Gram-positive and Gram-negative pathogens. Therefore, this study was aimed to evaluate the antibacterial activity of *M. charantia* seed extracts against selected microorganisms using the Kirby–Bauer disc diffusion method.

2 Materials and Methods

2.1 Cell maintenance and standardization

Bacterial cells used for the study were purchased from ATCC, USA. For the current study four Gram-positive (*Staphylococcus aureus* ATCC 29213, *Streptococcus mutans* ATCC 25175, *Bacillus cereus* ATCC 10876, and *Listeria monocytogenes* ATCC 19111) and four Gram-negative (*Acinetobacter baumannii* ATCC 19606, *Enterobacter cloacae* ATCC-BAA 1143, *Klebsiella pneumoniae* ATCC 10031, and *Salmonella enteritidis* ATCC 13076) bacterial cells were used. All bacterial cells were maintained on nutrient agar (Sigma-Aldrich, Germany). Prior to experimental procedure, the bacterial cells were inoculated into Mueller Hinton (MH) broth (Sigma-Aldrich, Germany) and left to incubate overnight at 200 rpm, 37°C to produce an overnight bacterial culture.

2.2 Plant Material and Extraction

Seed extracts of *Momordica charantia* originating from Malaysia were gifted from a Professor from UCSI University, Malaysia research laboratory. Plant extraction was done following protocol from Chaisawangwong and colleague (2013) with slight modifications.

The seed of *M. charantia* were macerated with 95%

methanol at a ratio of 2:8 of seed weight to methanol volume for 4 days under room temperature prior to mixture filtration. The process was repeated until a transparent filtrate was obtained. The collected filtrate was then dried at 40°C in a rotary evaporator to evaporate the excess solvent. The crude extract was placed in a desiccator tank, along with silica desiccant to absorb the remaining moisture. The drying crude extract was weighed daily until a constant weight was obtained to ensure all moisture has been removed.

2.3 Preparation of Inoculum

Bacterial inocula were prepared according to CLSI M02 (Performance Standards for Antimicrobial Disk Susceptibility Tests) protocol [11]. Fresh colonies from overnight cultures were suspended in sterile 0.85% saline solution and adjusted to match the turbidity of 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL.

2.4 Solvent Selection

Various solvents including methanol, ethanol, diethyl ether, dichloromethane, hexane, chloroform, and DMSO were evaluated for antibacterial interference and extract solubility. Sterile blank discs containing 20 μ L of each solvent were placed on inoculated agar plates. DMSO demonstrated optimal extract solubility without inhibitory effects on bacterial growth and was therefore selected as the extraction solvent.

2.5 Preparation of Seed Extract Concentrations

Serial dilution was performed to prepare extract concentrations ranging from 100 mg/mL to 800 mg/mL. Preliminary screening showed minimal activity at lower concentrations; therefore, higher concentrations (600 – 800 mg/mL) were further evaluated.

2.6 Antibacterial Disc Diffusion Assay

Antibacterial activity was assessed using the Kirby–Bauer disc diffusion method. Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions using sterile cotton swabs to obtain confluent growth.

Sterile 6 mm blank discs were impregnated with 20 μ L of *M. charantia* seed extract and placed onto inoculated agar plates. Doxycycline and tetracycline discs were used as positive controls, while blank discs containing DMSO served as negative controls.

The plates were incubated at 37°C and zones of inhibition were measured after 24, 48, 72, 96, and 120

hours. All experiments were performed in triplicate.

2.7 Statistical Analysis

Results were expressed as mean \pm standard deviation (SD) or standard error of mean (SEM). Statistical analysis was performed using GraphPad Prism software. Two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to determine significant differences between extract concentrations and incubation periods. A p-value <0.05 was considered statistically significant.

3 Results

3.1 Effect of Solvents on Selected Bacteria

None of the tested solvents demonstrated inhibitory activity against the selected bacterial strains. DMSO exhibited the best solubility profile for the seed extract and was therefore used throughout the study.

3.2 Antibacterial Activity Against Gram-Positive Bacteria

Staphylococcus aureus

No inhibitory activity was observed at concentrations below 600 mg/mL. At higher concentrations, *M. charantia* seed extract demonstrated measurable antibacterial activity against *S. aureus*. The highest inhibition zone was observed at 700 mg/mL with a mean diameter of 8.33 ± 0.33 mm at 24 and 48 hours. Activity remained relatively stable over 120 hours.

Streptococcus mutans

No antibacterial activity was observed against *S. mutans* at any concentration tested, including concentrations up to 800 mg/mL.

Bacillus cereus

Moderate antibacterial activity was observed against *B. cereus* at higher extract concentrations (500 – 800 mg/mL). Inhibition zones ranged between 7–9 mm depending on concentration and incubation period (Table 1).

Listeria monocytogenes

M. charantia seed extract demonstrated inhibitory activity against *L. monocytogenes*, particularly at all concentrations tested and highest inhibition at 700 mg/mL. Therefore, lower concentrations of the seed extracts were tested to observe for any significant effects at lower concentrations. There were inhibitions from concentrations between 40 – 80 mg/mL as well

(Table 2). Antibacterial activity remained relatively consistent throughout the incubation period.

3.3 Antibacterial Activity Against Gram-Negative Bacteria

Acinetobacter baumannii

No inhibition was observed at concentrations below 700 mg/mL. At 700 mg/mL, a consistent inhibition zone of approximately 7 mm was observed over 120 hours. Interestingly, antibacterial activity slightly decreased at 800 mg/mL, potentially due to reduced diffusion of the more viscous extract.

Enterobacter cloacae

The seed extract exhibited antibacterial activity against *E. cloacae* only at 800 mg/mL. Inhibition zones of approximately 6.67 ± 0.58 mm were observed after 48 hours of incubation.

Klebsiella pneumoniae and *Salmonella enteritidis*

No antibacterial activity was observed against *K. pneumoniae* or *S. enteritidis* at all concentrations tested.

4 Discussion

This study demonstrated that *Momordica charantia* seed extract possesses selective antibacterial activity against several Gram-positive and Gram-negative bacteria, supporting previous reports on the antimicrobial potential of medicinal plant extracts [4, 7]. The antimicrobial effect observed may be attributed to the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, triterpenoids, and glycosides that are known to possess antimicrobial properties.

Among the Gram-positive organisms tested, *S. aureus* exhibited the highest susceptibility to the seed extract. Similar findings have been reported in earlier studies evaluating *M. charantia* extracts against Gram-positive bacteria [9, 10]. This finding is consistent with previous studies reporting that *M. charantia* extracts are effective against Gram-positive bacteria due to differences in cell wall structure that facilitate penetration of phytochemicals.

The inhibitory activity against *A. baumannii* and *E. cloacae* is particularly noteworthy because these organisms are commonly associated with multidrug resistance and nosocomial infections. Although the inhibition zones observed were relatively small compared to standard antibiotics, the results indicate

Table 1. Zone of inhibition of *B. cereus* using different concentrations of *Momordica charantia* seed extract over 120 hours of incubation.

Disc	Concentration (mg/mL)	Mean Zone of Inhibition (mm)				
		24 h	48 h	72 h	96 h	120 h
1	500	8.667 ± 0.333	8.667 ± 0.333	8.667 ± 0.333	8.667 ± 0.333	8.333 ± 0.333
2	600	6.000	6.000	6.000	6.000	6.000
3	700	7.667 ± 0.333	7.667 ± 0.333	7.667 ± 0.333	7.000 ± 0.577	7.000 ± 0.577
4	800	8.333 ± 0.333	8.667 ± 0.333	8.667 ± 0.333	8.333 ± 0.333	8.333 ± 0.333
5	Blank disc	0.000	0.000	0.000	0.000	0.000
Ab	Tetracycline	16.667 ± 0.333	15.667 ± 0.333	15.667 ± 0.333	15.667 ± 0.333	15.000

Results are reported as mean ± SEM (mm).

Table 2. Zone of inhibition of *Listeria monocytogenes* using different concentrations of *Momordica charantia* seed extract over 120 hours of incubation.

Disc	Concentration (mg/mL)	Zone of Inhibition (mm)				
		24 h	48 h	72 h	96 h	120 h
1	20	0.000	0.000	0.000	0.000	0.000
2	40	15.000 ± 2.000	15.000 ± 2.000	15.000 ± 2.000	14.000 ± 2.000	14.000 ± 2.000
3	60	12.500 ± 0.500	12.500 ± 0.500	12.500 ± 0.500	12.500 ± 0.500	12.500 ± 0.500
4	80	20.500 ± 1.500	21.000	21.000	21.000 ± 1.000	20.500 ± 0.500
5	100	25.000 ± 2.082	24.667 ± 0.666	24.667 ± 0.666	24.667 ± 1.202	23.333 ± 1.333
6	200	21.333 ± 2.404	22.000 ± 2.309	22.000 ± 2.309	21.333 ± 2.404	21.333 ± 2.404
7	300	22.000 ± 1.732	22.000 ± 2.082	22.000 ± 2.082	22.000 ± 2.082	21.000 ± 1.732
8	400	19.000 ± 1.732	19.333 ± 0.882	20.333 ± 1.453	19.333 ± 0.882	19.000 ± 1.528
9	500	16.000 ± 2.000	16.500 ± 0.500	15.500 ± 1.500	15.500 ± 1.500	15.500 ± 1.500
10	600	29.333 ± 0.666	29.333 ± 0.666	29.333 ± 0.666	29.000 ± 1.000	29.000 ± 1.000
11	700	34.667 ± 1.453	34.667 ± 1.453	34.667 ± 1.453	36.000 ± 1.000	36.000 ± 1.000
12	800	24.667 ± 0.882	23.000 ± 1.000	23.333 ± 0.882	23.000 ± 0.577	23.000 ± 0.577
13	Blank disc	0.000	0.000	0.000	0.000	0.000
Ab	Tetracycline	29.333 ± 0.666	29.333 ± 0.666	28.667 ± 0.333	28.333 ± 0.333	28.000

Results are reported as mean ± SEM (mm).

the potential of *M. charantia* seed extract as a supplementary antimicrobial agent.

Interestingly, no antibacterial activity was observed against *S. mutans*, *K. pneumoniae*, and *S. enteritidis*. Resistance in these organisms may be attributed to intrinsic bacterial defense mechanisms such as efflux pumps, reduced membrane permeability, or biofilm formation.

The reduction in antibacterial activity observed at very high extract concentrations may be explained by poor diffusion of the viscous extract through the agar medium. This limitation is commonly encountered in disc diffusion assays involving crude plant extracts.

Overall, the findings support the ethnomedicinal use of *M. charantia* and highlight its potential for future antimicrobial drug development [2, 7]. However, further studies are necessary to isolate and characterize

the specific bioactive compounds responsible for the observed antibacterial activity.

5 Conclusion

Momordica charantia seed extract demonstrated selective antibacterial activity against several clinically important bacterial pathogens, particularly *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Acinetobacter baumannii*, and *Enterobacter cloacae*. The antibacterial activity was concentration-dependent and more pronounced at higher extract concentrations.

These findings support the traditional medicinal use of *M. charantia* and suggest that the plant may serve as a promising source of bioactive antimicrobial compounds. Further studies involving phytochemical characterization, determination of

minimum inhibitory concentration (MIC), and mechanistic studies are recommended.

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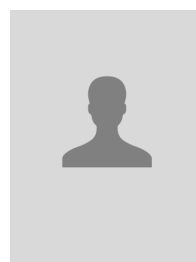
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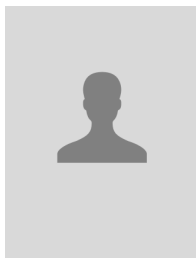
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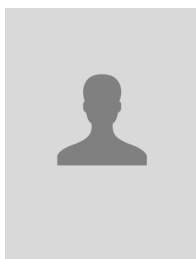
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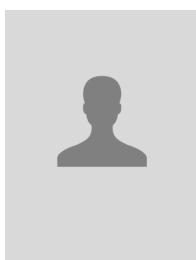
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