



## Research Article

### Evaluation of the Antibacterial Properties of Ethanolic and Aqueous Leaf Extracts of *Momordica charantia* against Some Bacterial Isolates

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#### ABSTRACT

*Momordica charantia* is known for its nutritional and medicinal properties, including antimicrobial and anti-inflammatory activities. This study evaluated the antibacterial activities of ethanolic and aqueous leaf extracts of *Momordica charantia* against the test isolates: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The leaves were washed, air-dried, pulverized and extracts were obtained. These extracts were then evaluated for antibacterial activity following standard microbiological protocols. The results showed that the zones of inhibition increased with an increase in concentration for both extracts. The ethanolic extract exhibited the highest inhibition against *K. pneumoniae* (16.00 mm), while *P. aeruginosa* showed 6.00 mm. The aqueous extract exhibited the highest inhibition against *K. pneumoniae* (14.00 mm) and the least against *E. coli* (6.00 mm). Overall, ethanolic extracts demonstrated greater antibacterial activity than aqueous extracts. For MIC, 100 mg/mL of ethanolic extract inhibited all test organisms, although *K. pneumoniae* showed sensitivity at 50 mg/mL. The aqueous extracts inhibited *K. pneumoniae* and *P. aeruginosa*, but not *E. coli* and *S. aureus*. MBC results revealed that ethanolic extracts exerted bactericidal effects on *S. aureus* and *P. aeruginosa*, while only bacteriostatic effects were observed against *E. coli* and *K. pneumoniae*. In contrast, only bacteriostatic effects were observed against *E. coli* and *K. pneumoniae*. These findings indicate that the ethanolic extract is more potent against the test isolates than the aqueous extract, which could be attributable to its rich phytochemical content, making it a promising candidate for further studies.

**Keywords:** Antibacterial properties; Bacterial isolates; Leaf extracts; *Momordica charantia*

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#### INTRODUCTION

Antimicrobial resistance among clinical bacterial pathogens is a growing public health challenge globally, prompting renewed interest in phytomedicinal agents as alternative therapies (Aldholmi *et al.*, 2024). *Momordica charantia*, commonly referred to as bitter melon or bitter gourd belong to the Cucurbitaceae family. It thrives in tropical and subtropical climates and is widely recognized for its medicinal properties. Traditionally, it is utilized across diverse cultures in Asia, Africa, and

South America. It has been employed in managing a variety of health conditions, including diabetes, infections, inflammation, and digestive issues (Bortolotti *et al.*, 2026). Its broad pharmacological activities have been verified in recent investigations (Pranavi *et al.*, 2024)

The leaves of *M. charantia* are a rich source of bioactive phytochemicals such as alkaloids, flavonoids, saponins, phenolics, tannins, and steroids, which are known to contribute to antimicrobial activity (Giwa *et al.*, 2024). Its therapeutic potential is

largely attributed to its complex composition of these phytochemicals, many of which have also demonstrated antioxidant activities.

Extraction with different solvents like water and ethanol play a crucial role in determining the yield, types, and potency of these compounds, since polar solvents (water) tend to extract hydrophilic constituents while ethanol can extract both polar and some non-polar phytochemicals (Abiola and Sunday, 2021; Giwa *et al.*, 2024).

Despite existing studies on the antimicrobial activity of *M. charantia*, there is limited comparative data evaluating the antibacterial efficacy of its leaf extracts using different solvents such as water (aqueous) and ethanol. This knowledge gap hinders informed decisions on optimal extraction methods for harnessing the plant's therapeutic potential.

This study therefore aims to systematically compare the antibacterial effects of ethanolic versus aqueous extracts of *Momordica charantia* leaves against some clinical bacterial isolates. By assessing differences in inhibition zones, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), the work seeks to identify which solvent yields the most potent extract and to contribute evidence toward developing plant-based antibacterial agents in the fight against drug-resistant pathogens. It is expected that both the ethanolic and aqueous leaf fractions would elicit antimicrobial properties, setting the stage for further studies.

## **MATERIALS AND METHODS**

### **Plant materials collection and preparation**

Fresh leaves of *Momordica charantia* were collected from a cultivated area at the Teaching and Research farm, University of Delta Agbor, Delta State. The leaves were taken to the laboratory where they were washed under running tap water to remove dirt, and air-dried at room temperature for four weeks until a constant weight was achieved. The dried leaves were subsequently ground into fine powder using a Binatone electric blender (BLG 450 model). The powdered sample was stored in an airtight container at room temperature until extraction. The preparation of the plant material followed the procedure described by Kilic and co-workers (Kilic *et al.*, 2023).

### **Obtaining plant extracts**

This followed the procedure (Wang *et al.*, 2023) with a little modifications. A total of 50 g of finely pulverized *Momordica charantia* leaf was separately submerged in 300 mL of distilled water and ethanol contained in sterile conical flasks. The mixture was

maintained at room temperature (25–28°C) for 72 hours with occasional shaking. The mixtures were filtered using Whatman No. 1 filter paper and the filtrates were concentrated using rotary evaporator maintained at 50°C to obtain the crude extract.

### **Inoculum Preparation**

Cultures of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* obtained from stored clinical culture collections in the Microbiology Laboratory, University of Benin, Benin City, were sub-cultured and standardized to 0.5 McFaland standard using Mueller-Hilton broth by transferring a loopful of the isolates into 3-4 mL of normal saline followed by proper shaking. The turbidity of the suspension was matched with that of 0.5 McFarland standards for sensitivity test as described by Clinical and Laboratory Standards Institute (CLSI, 2021).

### **Antibacterial Susceptibility Testing**

The antibacterial efficacy of both the aqueous and ethanolic leaf extracts of *Momordica charantia* was investigated utilizing the agar well diffusion technique according to Mekonnen and colleagues (Mekonnen *et al.*, 2022). Various concentrations of 12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL of the extracts were prepared using sterile distilled water as diluents to assess potential dose-dependent activity. Sterile nutrient agar was poured into Petri dishes and allowed to set. Each agar surface was then inoculated evenly with the standardized bacterial suspension using sterile swabs to establish a uniform lawn of bacterial growth. A sterile 6 mm cork borer was employed to punch wells into the agar medium. Into each well, different concentrations of the respective plant extracts were introduced under aseptic conditions. Ciprofloxacin (5 µg/disc), a broad-spectrum fluoroquinolone antibiotic, served as the positive control to validate the assay, while sterile distilled water functioned as the negative control to eliminate solvent-related interference in antimicrobial activity.

### **Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

The MIC was determined using the broth dilution method according to (Ngwai *et al.*, 2011) with a little modification. Briefly, two-fold serial dilutions of the extract was prepared in Mueller Hilton broth to obtain 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL. Each tube received 100 µL of bacterial suspension and extract. The plates were incubated at 37°C for 24 hours. The lowest concentration showing no visible signs of growth was recorded as the MIC.

MBC was determined by sub-culturing samples from tubes with no growth into nutrient agar and observed for bacterial growth after incubation.

#### Data Analysis

Statistical analysis was conducted using SPSS version 25. All tests were performed in triplicate to ensure reproducibility and accuracy. Data were recorded as Mean values  $\pm$  Standard Deviation. The results were statistically analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Post Hoc test to assess significant differences between the extracts and the drug. A *p-value* of less than 0.05 was considered statistically significant.

#### RESULTS

The antibacterial activity of both aqueous and ethanolic extracts of *Momordica charantia* is presented in Table 1. Results showed that the zone of inhibition increased with concentration for both extracts. The ethanolic extract exhibited the highest inhibition against *K. pneumoniae* (16.00 mm), while *P. aeruginosa* showed 6.00 mm. The aqueous extract exhibited highest inhibition against *K. pneumoniae*

(14.00 mm) and least against *E. coli* (6.00 mm). Overall, ethanolic extracts demonstrated greater antibacterial activity than aqueous extracts. At 50 mg/mL and 25 mg/mL, there was a statistically significant difference between the inhibition zones produced by ethanolic and aqueous extracts. At 100 mg/mL, the difference was not statistically significant. For MIC, 100 mg/mL of ethanolic extract inhibited all test organisms, although *K. pneumoniae* showed sensitivity at 50 mg/mL (Table 2). Aqueous extracts inhibited *K. pneumoniae* and *P. aeruginosa*, but not *E. coli* and *S. aureus*. The minimum bactericidal concentration (MBC) results (Table 3) revealed that ethanolic extracts exerted bactericidal effects on *S. aureus* and *P. aeruginosa*, while only bacteriostatic effects were observed against *E. coli* and *K. pneumoniae*. In contrast, only bacteriostatic effects were observed against *E. coli* and *K. pneumoniae*. These findings indicate that ethanolic extract demonstrates high inhibitory effects against test isolates, bactericidal effects against *S. aureus* and *P. aeruginosa* as compared to aqueous extract.

**Table 1: Diameter of zone of inhibition (mm) of aqueous and ethanolic extracts of *Momordica charantia* leave extract**

| Test organism                 | Extract   | ZONES OF INHIBITIONS (mm) |                |                |                | CPX (control) |
|-------------------------------|-----------|---------------------------|----------------|----------------|----------------|---------------|
|                               |           | 100 mg/mL                 | 50 mg/mL       | 25 mg/mL       | 12.50 mg/mL    |               |
| <i>Escherichia coli</i>       | Ethanolic | 10.0 $\pm$ 1.0            | 7.0 $\pm$ 2.0  | 0.0 $\pm$ 0.0  | 0.0 $\pm$ 0.0  | 20            |
|                               | Aqueous   | 6.0 $\pm$ 1.0             | 0.0 $\pm$ 0.0  | 0.0 $\pm$ 0.0  | 0.0 $\pm$ 0.0  |               |
| <i>Staphylococcus aureus</i>  | Ethanolic | 12.0 $\pm$ 1.0            | 9.0 $\pm$ 1.0  | 6.0 $\pm$ 1.0  | 0.0 $\pm$ 0.0  | 16            |
|                               | Aqueous   | 9.0 $\pm$ 1.7             | 6.0 $\pm$ 1.0  | 0.0 $\pm$ 0.0  | 0.0 $\pm$ 0.0  |               |
| <i>Pseudomonas aeruginosa</i> | Ethanolic | 6.0 $\pm$ 0.0             | 9.0 $\pm$ 2.6  | 0.00 $\pm$ 0.0 | 0.00 $\pm$ 0.0 | 14            |
|                               | Aqueous   | 10.0 $\pm$ 1.0            | 4.0 $\pm$ 1.0  | 0.00 $\pm$ 0.0 | 0.00 $\pm$ 0.0 |               |
| <i>Klebsiella pneumoniae</i>  | Ethanolic | 16.0 $\pm$ 1.0            | 12.0 $\pm$ 2.0 | 8.0 $\pm$ 1.0  | 0.00 $\pm$ 0.0 | 22            |
|                               | Aqueous   | 14.0 $\pm$ 2.0            | 9.0 $\pm$ 2.6  | 0.00 $\pm$ 0.0 | 0.00 $\pm$ 0.0 |               |

CPX: Ciprofloxacin

**Table 2: Minimum inhibitory concentration (MIC) of *Momordica charantia* against test isolates.**

| Isolates                      | Ethanol (extract) | Aqueous (extract) |
|-------------------------------|-------------------|-------------------|
| <i>E. coli</i>                | 100mg/ml          | -                 |
| <i>Klebsiella pneumoniae</i>  | 50mg/ml           | 100mg/ml          |
| <i>Staphylococcus aureus</i>  | 100mg/ml          | -                 |
| <i>Pseudomonas aeruginosa</i> | 100mg/ml          | 100mg/ml          |

(-) indicates no inhibition observed at the tested concentrations

**Table 3: Minimum Bactericidal Concentration (MBC) of *Momordica charantia* (Bitter Gourd) Extracts on Bacterial Isolates**

| Isolates                      | Ethanolic sExtract (Concentration) | Activity       | Aqueous Extract (Concentration) | Activity       |
|-------------------------------|------------------------------------|----------------|---------------------------------|----------------|
| <i>Escherichia coli</i>       | 100 mg/ mL                         | Bacteriostatic | 100 mg/mL                       | —              |
| <i>Klebsiella pneumoniae</i>  | 50 mg/ mL                          | Bacteriostatic | 100 mg/mL                       | Bacteriostatic |
| <i>Staphylococcus aureus</i>  | 100 mg/ mL                         | Bactericidal   | 100 mg/mL                       | —              |
| <i>Pseudomonas aeruginosa</i> | 100 mg/mL                          | Bactericidal   | 100 mg/mL                       | Bacteriostatic |

#### DISCUSSION

The present study provides valuable insights into the comparative antibacterial efficacy of ethanolic and

aqueous leaf extracts of *Momordica charantia* against common clinical bacterial pathogens. The results indicate that the ethanolic extract consistently

demonstrated superior antibacterial activity compared to the aqueous extract and this is in tandem with previous workers (Akpotu *et al.*, 2017; Ibitoye *et al.*, 2022).

The agar well diffusion results revealed a clear dose-dependent relationship, with increasing extract concentrations correlating to larger inhibition zones. Notably, *Klebsiella pneumoniae* showed the highest sensitivity to the ethanolic extract, while *E. coli* was the least sensitive. In contrast, the aqueous extract displayed weaker inhibitory effects across all organisms, with *E. coli* again being the least susceptible.

The superior performance of the ethanolic extract over the aqueous extract can be linked to the ethanol's capacity to solubilize a broader spectrum of phytochemicals, including both polar and moderately non-polar compounds such as flavonoids, alkaloids, and saponins (Nourbakhsh *et al.*, 2022; Maran *et al.*, 2022).

The MIC results further corroborate the superior potency of the ethanolic extract. *K. pneumoniae* showed sensitivity at 50 mg/mL, while other pathogens required 100 mg/mL. The aqueous extract inhibited only *K. pneumoniae* and *P. aeruginosa* at 100 mg/mL and failed to inhibit *E. coli* and *S. aureus*, indicating a narrower antibacterial spectrum.

MBC findings showed that the ethanolic extract exhibited bactericidal activity against *S. aureus* and *P. aeruginosa*, while it was bacteriostatic against *E. coli* and *K. pneumoniae*. In contrast, the aqueous extract demonstrated only bacteriostatic effects and failed to exhibit any bactericidal effect on three out of the four organisms tested.

These bacteriostatic and bactericidal distinctions are clinically important. A bactericidal agent like the ethanolic extract is especially valuable in managing life-threatening infections in immunocompromised patients. This echoes the conclusions of Maran *et al.* (2022) and Giwa *et al.* (2024) that phytochemicals like tannins and alkaloids may possess selective bactericidal properties.

These findings are consistent with the results of Abiola and Sunday (2021), reported greater antibacterial activity in ethanolic extracts of *M. charantia* compared to aqueous forms. Similarly, Ude *et al.* (2022) observed that while both solvent systems extracted bioactive components with antibacterial effects, ethanol produced extracts with lower MIC values and broader inhibition spectra. Olafimiha and Sunday (2021) also documented similar outcomes, reinforcing ethanol's efficiency in extracting bioactive phytochemicals.

Although the extracts did not surpass ciprofloxacin (used as the positive control), the significant zones of inhibition, particularly against multidrug-resistant *K. pneumoniae*, are encouraging. This supports earlier findings by Ndlovu *et al.* (2023), highlighted the urgent need for plant-derived antimicrobials due to increasing resistance among Enterobacteriaceae.

However, the current study focused only on aqueous and ethanolic extracts and did not assess the efficacy of other extraction solvents such as methanol, acetone, chloroform, or ethyl acetate. Studies have shown that methanol can extract a distinct set of phytochemicals that may not be effectively captured by water or ethanol alone (Ezekiel *et al.*, 2016). Therefore, while ethanol demonstrated better results in this context, it is not sufficient to conclude definitively that ethanol is the "best" solvent for extracting antimicrobial compounds from *M. charantia* leaves.

Moreover, variations in extraction efficiency and antibacterial activity across different solvents are often due to differences in solvent polarity, phytochemical solubility, and the targeted bioactive compounds. The methanolic extracts have been shown to contain higher concentrations of phenolic acids and flavonoids, which could influence antibacterial potency (Giwa *et al.*, 2024). Hence, solvent-specific extraction plays a critical role in determining the phytochemical profile and the therapeutic potential of plant extracts.

## CONCLUSION

This study affirms the antibacterial potential of *Momordica charantia* leaf extracts, particularly the ethanolic extract, which exhibited superior inhibitory and bactericidal activity compared to the aqueous extract. The enhanced activity of the ethanolic extract is likely due to its ability to solubilize a wider range of bioactive compounds, including flavonoids and saponins. However, given that only two solvents (water and ethanol) were used in the comparative evaluation, it would be hasty to conclude that ethanol is the most effective solvent for extracting antibacterial compounds from *M. charantia*.

Future studies should incorporate other solvents of varying polarities such as methanol, chloroform, acetone, and ethyl acetate, and possibly employ sequential or Soxhlet extraction to fully characterize the phytochemical and antimicrobial profiles. Additionally, further work should aim to isolate and characterize the specific active constituents responsible for antibacterial activity, evaluate potential synergistic interactions with conventional

antibiotics, and conduct in vivo efficacy and toxicity assessments to validate therapeutic potential.

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### **Conflict of interest**

The authors wish to declare that there are no conflict of interests.

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