ISSN: 3116-3475
International Journal
of Education, Research, and Innovation Perspectives
Volume 2 Issue 6 (June 2026)

Inhibitory Potential of Momordica Charantia Fruit Extract Against Microbial Isolates of Urinary Tract Infection

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Date Submitted:
June 12, 2026

Date Accepted:
June 12, 2026

Date Published:
June 16, 2026

DOI:
10.5281/zenodo.20721365

ABSTRACT

Urinary Tract Infection (UTI) is a prevalent bacterial infection that carries serious health risks on the present global scale due to its increasing morbidity rates and antibiotic resistance. This study was designed to assess the inhibitory effect of the fruit extract of Momordica charantia (bitter gourd) on bacteria causative agents of UTIs such as Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa collected from UTIs patients. This study adopted an experimental research design, which involved using the Kirby-Bauer disc diffusion technique. The concentration of methanol extracts used in this study was 50%, 75%, and 100%. It was observed that there was no inhibition recorded at 50% concentration, while there was a significant zone of inhibition at both 75% and 100% concentrations ($p = 0.000 < 0.05$). These findings suggest that M. charantia fruit methanol extract possesses inhibitory properties and can serve as a candidate for controlling bacterial growth in UTI cases.

Keywords: *Momordica charantia, urinary tract infection, antibacterial property, kirby-bauer, zone of inhibition*

INTRODUCTION

Urinary Tract Infection (UTI) continues to be one of the most common bacterial infections and represents a serious public health problem worldwide, having affected more than 150 million people per year. In 2019, it was reported that there were over 236,790 deaths caused by UTIs. This problem also affects the Philippines, where, according to the Epidemiology Bureau of the Department of Health, infectious diseases represent eight out of ten leading causes of morbidity, with UTIs being the third most frequent cause of morbidity.

The pathogens causing UTIs include mostly Gram-negative bacteria, which represent 80% of all UTIs; these pathogens are *Escherichia coli*, *Klebsiella spp.*, and *Pseudomonas spp.* Uropathogens usually penetrate by the ascending pathway, infecting the vaginal introitus and disturbing the normal microflora, thus causing either acute or recurrent UTI. Considering the high incidence rate and high likelihood of recurrence, anti-microbial medications have been prescribed to patients quite often. As a result, their inappropriate use has led to increased antimicrobial resistance against common medications, such as ampicillin and gentamicin. Hence, new antibiotics are needed to address this growing problem.

The usefulness of plants as medicinal products dates back decades. Approximately 50% of drug compositions come from herbs. *Momordica charantia L.* (bitter gourd, ampalaya) is one of the tropical

ISSN: 3116-3475
International Journal
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vines noted for its medicinal value. Aside from its documented hypoglycemic action, several bioactive compounds in it include triterpenes, proteins, steroids, alkaloids, and phenolic substances responsible for its antioxidant, antitumor, and antimicrobial functions. Previous studies showed antibacterial actions of its leaves against strains of Escherichia coli and Pseudomonas; there is yet no evidence on the inhibitory power of the fruit extract against pathogens causing UTIs.

This study is based on Germ Theory stating that some specific organisms cause particular diseases. Penicillin, a natural compound with antimicrobial function, serves as a background for the proposal. The hypothesis here focuses on *M. charantia* fruit extract containing bioactive compounds like saponins and flavonoids. They can be responsible for its bactericidal effects against uropathogenic bacteria. In this study, a methanol-based extraction method was used, shown to work better than hexane or acetone. *M. charantia* fruit extract will be assessed for possible inhibitory actions to prove the potentiality of its use in inhibiting growth of harmful bacteria. This will lead to determination of the antibacterial effect of various concentrations of the extract on strains of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

METHODS

Research Design

The study utilized an experimental research design, focusing on a scientific approach to establish a cause-and-effect relationship between the independent variable (crude extract concentrations) and the dependent variable (zones of inhibition).

Research Locale and Sampling Technique

Microbial isolates were collected from the urine samples of 30 outpatients at a tertiary hospital in Los Baños, Laguna. Pathogens were successfully isolated from 8 patients, including *E. coli* (5 patients), *K. pneumoniae* (2 patients), and *P. aeruginosa* (1 patient)

Instrumentation

The experiments were performed by using an extensive array of laboratory equipment that included a biosafety cabinet, an incubator, and an autoclave, all of which were in a tertiary clinical laboratory in Laguna. Selective isolation of Gram-negative bacteria, namely *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, as well as their identification by means of a number of biochemical tests, was achieved by means of MacConkey agar along with a variety of biochemical identification media, namely Lysine Iron Agar (LIA), Simmons Citrate Agar (SCA), Sulfide-Indole-Motility (SIM), Triple Sugar Iron Agar (TSI), and Urease Agar (UA). Antibiotic susceptibility was tested using the Kirby-Bauer disc diffusion test on Mueller-Hinton (MH) agar plates, with inhibitory zones measured digitally with a caliper according to CLSI guidelines.

The validity of the procedure was confirmed by conducting sterility tests to make sure that there were no live organisms in all of the media tested prior to its application for growing the microorganisms. Furthermore, the bacterial inoculum was prepared by reaching the 0.5 McFarland standard level (1×108 to 2×108 CFU/mL). Statistical analysis of results based on triplicate experiments was performed using Two-Way ANOVA with Post-Hoc Tukey tests and t-tests at $\alpha = 0.05$ to prove statistical significance among different concentrations of extracts as compared with the positive control group (Levofloxacin).

Analysis of Data

The experiment findings were summarized and analyzed statistically using a significance level of $\alpha = 0.05$. In order to determine if there was any statistical difference in the zones of inhibition of the various concentrations of the M.charantia fruit extract, as well as the positive and negative controls, for the three bacterial isolates (E.coli, K.pneumoniae, and P.aeruginosa), a Two-way ANOVA (analysis of variance) with Post-hoc Tukey test was used. A t-test was also performed to find the mean of the zones of inhibition in each trial. The purpose of this test was to provide a probability under the null hypothesis.

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Ethical Considerations

In line with putting ethical considerations first, patient's rights and informed consent are of utmost priority in conducting the research. Every one of the 30 patients who participated in the experiment was given a waiver and consent form, stating that their urine samples will be used for further studies to test the antimicrobial effect of the material. Every communication between the Ancillary Manager and the CEO of the tertiary hospital at Los Baños, Laguna was duly approved before the experiment was conducted.

RESULTS AND DISCUSSION

Antibacterial Activity Against *Escherichia coli*

Table 1 presents the measured zones of inhibition across three trials, comparing the 100%, 75%, and 50% concentrations against both positive (Levofloxacin) and negative (methanol) controls.

Table 1. *Result of zone of inhibition of Momordica charantia extract against Escherichia coli*

Trial	Positive Control	Negative Control	1.0mg/ul (100%)	0.75mg/ul (75%)	0.50mg/ul (50%)
1	23.78 mm	6 mm	14.68 mm	9.44 mm	6 mm
2	23.39 mm	6 mm	13.89 mm	9.37 mm	6 mm
3	24.98 mm	6 mm	14.56 mm	9.24 mm	6 mm
Average	**24.05 mm**	**6 mm**	**14.37 mm**	**9.35 mm**	**6 mm**

Note: *No zone of inhibition: 6mm.*
CLSI 2022 guidelines: ≥ 21mm (S), 17-20mm (IS), ≤ 16 ®.

As seen from the results, the 0.50 mg/µL (50%) solution did not inhibit bacterial growth at any point during the experiment. This result may be explained by the impermeability of the outer membrane of *E. coli* bacteria and efflux pumps that decrease intracellular antibiotic concentrations. However, despite the lack of activity of the lower concentration, the average zones obtained for 75% (9.35 mm) and 100% (14.37 mm) solutions fall under the resistant range of the CLSI guidelines with a maximum of 16 mm.

Antibacterial Activity Against *Klebsiella pneumoniae*

Table 2 details the results for each trial and concentration, highlighting the comparative performance of the extract against the standardized controls

Table 2. *Results of zone of inhibition of Momordica charantia extract in Klebsiella pneumoniae.*

Trial	Positive Control	Negative Control	1.0mg/ul (100%)	0.75mg/ul(75%)	0.50mg/ul (50%)
1	22.66mm	6 mm	19.58mm	17.03mm	6 mm
2	24.50mm	6 mm	18.88mm	15.77mm	6 mm
3	22.66mm	6 mm	18.13mm	15.89mm	6 mm
Average	**23.27mm**	**6 mm**	**18.86mm**	**16.23mm**	**6 mm**

Note: *No zone of inhibition: 6mm.*
CLSI 2022 guidelines: ≥ 21mm (S), 17-20mm (IS), ≤ 16 ®

Similar with the Escherichia coli sample, the 50% concentration of the isolate failed to show any antibacterial action. At higher concentrations, though, there was a marked rise in sensitivity of the bacterial strain to the compound tested. The inhibition zone for the 100% concentration was recorded to be 18.86 mm and classified as Intermediately susceptible (17–20 mm) based on CLSI criteria. This antimicrobial activity is also supported by the identification of seed polysaccharides in the fruit extract. Despite all this, however, the crude extract could not match the strength of Levofloxacin, which might be explained by the presence of contaminants in the extract itself.

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International Journal
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Antibacterial Activity Against *Pseudomonas aeruginosa*

Table 3 summarizes the zones of inhibition recorded for this pathogen, which is known for its high adaptability and resistance mechanisms.

Table 3. *Results of zone of inhibition of Momordica charantia extract in Pseudomonas aeruginosa.*

Trial	Positive Control	Negative Control	1.0mg/ul (100%)	0.75mg/ul (75%)	0.50mg/ul (50%)
1	37.16mm	6 mm	15.69mm	10.60mm	6 mm
2	38.88mm	6 mm	13.53mm	9.54mm	6 mm
3	39.93mm	6 mm	15.95mm	10.62mm	6 mm
Average	**38.66mm**	**6 mm**	**15.06mm**	**10.25mm**	**6 mm**

Note: *No zone of inhibition: 6mm*
CLSI 2022 guidelines: ≥ 22mm (S), 15-21mm (IS), ≤ 14 ®.

The results demonstrate that while the 75% concentration remained in the resistant category, the 100% concentration achieved intermediate susceptibility with an average zone of 15.06 mm. The resistance profile of P. aeruginosa is historically challenging due to its low membrane permeability and its ability to produce antibiotic-inactivating enzymes. Per CLSI standards, an intermediate result implies potential clinical applicability in specific body sites where the extract can reach higher concentrations or in situations where higher dosages are utilized.

Comparative Statistical Analysis of Extract Concentrations

Table 4 presents the results of the ANOVA testing used to identify significant differences in antibacterial property

Table 4. *Antibacterial Property among the Different Concentration of Methanol Fruit Extract against the Isolated Bacteria*

Different Concentration	***Mean***	***F-test***	***p-value***	***Interpretation***
Momordica charantia extract in Escherichia coli				
Positive control (1)	24.05	981.503*	0.000	Significant
Negative control (2)	6.00			
100% (3)	14.38			
75% (4)	9.35			
50% (5)	6.00			
Momordica charantia extract in Klebsiella pneumoniae				
Positive control (1)	23.27	425.508*	0.000	Significant
Negative control (2)	6.00			
100% (3)	18.86			
75% (4)	16.23			
50% (5)	6.00			

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Momordica charantia *extract in* Pseudomonas aeruginosa				
Positive control (1)	38.66	680.093*	0.000	Significant
Negative control (2)	6.00			
100% (3)	15.06			
75% (4)	10.25			
50% (5)	6.00			
**Significant @ 0.05*				

The statistical analysis reveals a significant difference ($p = 0.000 < 0.05$) in the antibacterial property across all isolates when comparing different concentrations and the positive control. The maximum activity observed in the methanolic extract is attributed to its high content of alkaloids, glycosides, and volatile oils, as well as molecules such as MAP30 found in the seeds. These findings provide sufficient evidence that *M. charantia* fruit extract possesses a bacteriostatic property, the ability to inhibit growth making it a viable candidate for controlling common UTI uropathogens

CONCLUSION

Firstly, the primary objective of this study is to determine the potential of M. charantia fruit extract as an inhibitor of clinical uropathogens. Secondly, the findings of this study are anchored in the Germ Theory and the history of antibiotics. According to the results, the methanol fruit extract is bactericidal. However, it has strict concentration dependency and different activities against various bacteria. For instance, the methanol extract displayed considerable zones of inhibition at 75% and 100% concentrations ($p = 0.000$). Consequently, these results provide empirical support for the claim that the M. charantia plant inhibits *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

Secondly, the findings demonstrate the resistance of *E. coli* at various concentrations according to the CLSI criteria. At a molecular level, this resistance can be attributed to the impermeable nature of the *E. coli* outer membrane and efflux pumps that reduce the antibacterial agent levels within the organism. However, the intermediate susceptibility of *K. pneumoniae* and *P. aeruginosa* at the 100% concentration implies that the active components of the fruit, such as alkaloids, glycosides, and volatile oils, are effective in overcoming these barriers. This assumption is justified by past studies, which show that the seeds and pulp of bitter gourd have molecules with broad antimicrobial activities.

In comparison to the positive control, the crude extract exhibited significantly less efficacy than that of Levofloxacin. The reduced level of efficiency may be explained by the crude nature of the sample and its consequent purity, leading to reduced levels of antibacterial activity compared to synthetically-made and standardized drugs. Furthermore, while growth inhibition occurred during testing, the crude extract mostly exhibited bacteriostasis and not bactericidal activity since it was unable to eliminate bacteria in high concentrations.

The significance of these results is that there could be a foundation on which to develop organic drug treatments against increasing antibiotic resistance around the globe. By demonstrating that the extracts from M. charantia can inhibit the growth of bacteria, it becomes clear that there could be the possibility for drug discovery and alternative medical practices that may eventually bring down the costs of using synthetic medicines.

Further studies may wish to purify the samples and use chromatography techniques to standardize the samples and their active ingredients. It would also be beneficial to explore the synergies that can occur when *M. charantia* is combined with other medicinal plants. Molecular tests should also be conducted to

assess how effective the active compounds in the fruits are against multidrug-resistant bacteria. Finally, the use of increased dosages and alternative extraction solvents may yield further insight into solubility.

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