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Momordica charantia Schaefer Leaf Extract Antibacterial Efficacy, Phytochemical Screening, and Toxicological Studies

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Momordica charantia, also known as Bitter melon or Bitter gourd, is a Cucurbitaceae plant that is widely grown in tropical and subtropical areas. It has a wide range of applications, including antibacterial, antidiabetic, anthelmintic, and antioxidant. *Momordica charantia's* antibacterial activity was tested against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis* using standard techniques. Antibacterial activity was tested using agar-well diffusion techniques. The results showed that ethanol and n-hexane extracts inhibited the growth of all of the bacteria tested at a dosage of 100 mg/ml, although with different susceptibility. The diameter of zones of inhibition obtained ranged from 10mm -15.1mm and 8.2mm - 14 mm for ethanol and n-hexane extracts respectively. For both ethanol and n hexane extracts, the minimum inhibitory concentration (MIC) ranged from 30 to 40 mg/ml. For both ethanol and n-hexane extracts, the minimum bacteriocidal concentrations varied from 30 to 40 mg/ml. Tannin, saponin, alkaloids, and polyphenol were found in the phytochemical screening results. The histology of the liver and large intestine of the seemingly healthy albino rats fed the leaf extract was normal. *Momordica charantia* may be utilised to treat infections and disorders caused by these bacteria, according to the findings of this study.

Keywords: Momordica charantia; antibacterial; histological.

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1. INTRODUCTION

Medicinal plants have been employed as phytotherapeutics all across the world since antiquity. In general, medicinal plants' bioactive compounds are secondary metabolites that have a variety of pharmacological effects and are employed as ingredients in modern medications. In the search for bioactive molecules, plants are a promising source of natural chemicals [1]. According to reports, traditional medicines are the primary source of medication for 80% of the world's population [1,2]. Plants have been used to treat a variety of infections and disorders. As a result, many plants have become key drug sources, and pharmaceutical companies have begun to view traditional medicine as a source of bioactive molecules that can be employed in the development of novel medications.

According to reports, traditional medicines are the primary source of medication for 80% of the world's population [1]. The clinical success of plant-based medications has reignited interest in medicinal plant research as a source of possible new drugs. *Momordica Charantia* is a tropical and subtropical plant of the *Cucurbitaceae* family that is extensively dispersed around the world [3] Bitter gourd leaves (*Momordica charantia*) have long been used as a food and medication [3]. Since it grows in tropical regions such as India, Malaya, China, tropical Africa, the Middle East, America, and Thailand, it has a variety of names [4]. Bitter gourd vines flower in about 30 days and produce full fruits in about 20 days when propagated by seed. One example is bacterial resistance. Bacterial resistance is expected to be one of the biggest causes of death in the globe by 2050, according to experts [5]. The herb has a long history of medicinal use among Amazonian indigenous peoples. Rural dwellers prefer traditional remedies, according to Mbuni et al. [6] because of their proximity to traditional healers and the fact that the healers are familiar with their culture and surroundings, as well as their patients. A leaf tea can be used to treat diabetes, colic, ulcers, wounds, and infections topically, internally and externally for worms and parasites, emmenagogue, and antiviral for measles, hepatitis, and febrile disorders. Antidotal, antipyretic, agreeable, and stomachic effects are all present [7].

It's also used to promote digestion, metabolism, blood circulation, immunity, and vigour in Asian and African traditional treatments. Bitter guard decreases fever, blood impurities, and jaundice, according to Ayurveda, an Indian system of medicine. Furthermore, this vegetable aids in the treatment of liver disorders, skin maladies, and other respiratory problems [8]. Many plant species have been screened and tested for antibacterial qualities, according to ethnomedical sources, however the great majority of plants have not been fully examined and analyzed [9] The present study aims to investigate the antibacterial activity, phytochemical screening, and toxicological examinations of the leaves of *M. charantia* against various bacteria, taking into account the huge potentiality of plants as sources of antimicrobial medications.

2. MATERIALS AND METHODS

2.1 Materials

Momordica charantia leaves, weighing balance, mechanical grinder, incubator, oven, petri-dishes, sterile filter paper, rotary evaporator, cork borer, test organisms (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus*), conical flask, beaker, polythene bag, autoclave, nutrient agar.

2.2 Collection of Sample

Momordica charantia leaves were taken from a farm in the Omo-Owo district of Offa, Kwara State. It was botanically identified, and a botanist from the Department of Science Laboratory Technology, Federal Polytechnic Offa, Kwara State, Nigeria, confirmed the identification. The leaves were collected in a polythene bag and delivered to the experiment site right away.

2.3 Sample Preparation

The *Momordica charantia* leaves were cleaned and air dried at room temperature in the shade. Prior to the antibacterial test, the plant material was dried and powdered using a mortar and pestle, then stored in a moisture-free, airtight container at room temperature.

2.4 Preparation of Ethanol and N-Hexane Extract

In various conical flasks, 100 g of dry powdered sample was soaked in 500 ml of 70 percent ethanol and n-hexane. Each flask was covered with cotton wool, coated in aluminum foil, and vigorously shaken for 48 hours at room

temperature at 5-hour intervals. The crude extract was sieved using muslin cloth and Whatman no 1 filter paper after 48 hours. A rotary evaporator was used to evaporate the filtrate to dryness. Until needed, the dried extracts were kept in airtight sample bottles.

2.5 Reconstitution of the Extract

For antibacterial testing, 100 and 200 mg of crude extract were dissolved in 1 ml of distilled water, respectively, to obtain concentrations of 100 and 200 mg/ml.

2.6 Sterilization Techniques

Glasswares: Before usage, all glassware was rinsed with soapy water and sterilized in a 160^0C oven for 2 hours.

Culture media: All media were sterilized at 1210C for 15 minutes according to the manufacturer's instructions.

2.7 Standardization of the Organisms

Using the McFarland standard, the organisms were standardized. In order to determine bacterial density, McFarland tube no. 0.5 was made by combining 0.05ml of 1.175 percent barium chloride (BaCl2) with 9.95 ml of 1 percent sulphuric acid (H2SO4) in distilled water. The tube was sealed and used to compare the bacterial suspension to a standard whenever it was necessary.

2.8 Sources of Test Microorganisms

From General Hospital Ilorin, Kwara State, and Lautech Teaching Hospital Osogbo, Osun State, Nigeria, pure cultures of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus* were collected.

2.9 Antibacterial Activities

The agar-well diffusion method was used to test the antibacterial activity of crude extract. Before being used, all test organisms were cultivated for 24 hours in nutrient agar and standardized to 0.5 McFarland standards (10^8 cfu/ml). The organism was placed on a Mueller hinton agar plate and inoculated. In each agar plate, a sterile cork borer with a diameter of 6mm was used to create four wells. The extract was divided into three wells: 100mg/ml, 200mg/ml, and sterile water as a negative control. In the fourth well, chloramphenicol served as a positive control. In an incubator, all plates were incubated at 37°C for 24 hours. Following a 24-hour incubation period, the plates were examined for zones of inhibition.

2.10 Determination of Minimum Inhibitory Concentration (MIC)

The Akinpelu and Kolawole method [10] was used to ascertain the plant extract's MIC (2004). In a nutshell, concentrations of the extract of 10.0, 20.0, 40.0, and 50.0 mg/ml were made and added to each test tube containing 9 ml of nutritional broth. In test tubes containing agar broth and extract, 1 mL of the 18-hour standardized organism was also added. At 370 degrees Fahrenheit, all of the test tubes were incubated for 24 hours. The MIC was determined by using the lowest concentration of extract that prevented observable growth in the broth. For each test organism, the extracts' MIC was ascertained.

2.11 Determination of Minimum Bactericidal Concentration (MBC)

The Spencer and Spencer method was used to assess the MBC of plant extracts [10]. One millimeter of broth was taken from the tubes that did not develop in the MIC assay and sub cultured on freshly prepared nutrient agar before incubation at 370C for 48 hours. On a new set of agar plates, the MBC was chosen as the extract concentration that induced no growth.

2.12 Phytochemical Screening

The qualitative analysis and extractions were carried out using the most standard and dependable procedures [10].

2.13 Experimental Animals

In the botanical garden of Federal Polytechnic Offa, Kwara State, Nigeria, eighteen [11] Wister rats of the same sex weighing between 140 and 250g were maintained in a cage.

2.14 Histopathology

The liver and big intestine of the seemingly healthy albino rats were examined histopathologically.

Assessing the Health Status of the Rats Prior to Study: Prior to the investigation, the rats were observed for signs and symptoms of infection such as loss of appetite, weakness, conjunctivitis, stool texture etc for seven days.

Determination of microbial loads and types in the faeces of apparently healthy albino rats before the commencement of the research work: The faeces of the albino rats were examined before the commencement of the research work, because their faeces serves as an indicator of the microflora present in their gastrointestinal tract.

Determination of the infective dose of (*Escherichia coli and Pseudomonas aeruginosa)* on albino rat: The infectious dose was determined according to the method described by [10].

Infection of apparently healthy albino rats with the determined infective dose of *(Escherichia coli and Pseudomonas aeruginosa):* Six apparently healthy albino rats were orogastrically administered with the infectivity dose determined, while three apparently healthy albino rats served as the control. The rats were then observed for signs and symptoms of illness such as weakness, loss of appetite, informed stool, scattered fur etc. Their stool was taken for microbiological examination after 24 hours for five days to determined their microbial types and loads.

Treatment of infected albino rats with crude plant extract of momordica charantia: After infection sets in the animals were divided into three groups (A, B & C). Group A were the animals administered with *Escherichia coli,* Group B were animals administered with *Pseudomonas aeruginosa* and Group C animals served as control. Both group A and B were treated with crude plant extracts of Momordica (1ml daily) for 3 days, while group C was left as the control. The rats were observed for signs and symptoms of illness such as weakness, loss of appetite, unformed stool, scattered fur etc

Toxicological Studies: The liver and the large intestine of the healthy albino rats were investigated histopatholically.

2.15 Statistical Analysis

The data received from antibacterial actions were statistically analyzed using a social science statistical software (SPSS).

3. RESULTS

3.1 Antibacterial Activities of *Momordica charantia* leaf Extract Prepared at 100mg/ml

The diameter of zone of inhibition of the ethanolic leaf extract of *Momordica charantia* against the tested bacteria ranged from 10.0 – 15.1mm, while that of n Hexane ranged from 8.2 – 14.0 mm respectively. The sterile water that was used as a negative control showed no antibacterial activities, while chloramphenicol antibacterial activities ranged from 11.0 – 17.9 mm respectively.

3.2 Antibacterial Activities of *Momordica charantia* Leaf Extract Prepared at 200mg/ml

The diameter of zone of inhibition of the ethanolic leaf extract of *Momordica charantia* against the tested bacteria ranged from 11.2 – 17.1mm, while that of n Hexane ranged from 9.4 – 14.5 mm respectively. The sterile water that was used as a negative control showed no antibacterial activities, while chloramphenicol antibacterial activities ranged from 11.2 – 18.0 mm respectively.

3.3 Minimum Inhibitory Concentration of the Leaf Extract of *Momordica charantia*

The minimum inhibitory concentration for both ethanolic and n Hexane extract ranged from 30 – 40mg/ml.

Table 1. Antimicrobial activities of *Momordica charantia* extract prepared at 100mg/ml

	Diameter of zones of inhibition in mm			
Test Organisms	**Ethanol Extract**	**n hexane extract**	**Water**	**Chloramphenicol**
Staphylococcus aureus	10.0 ± 1.0	9.0 ± 0.6	-	13.0 ± 0.2
Bacillus subtilis	14.2 ± 0.4	13.3 ± 0.2	-	12. 3 ± 0.6
Escherichia coli	15.1 ± 0.3	14.0 ± 0.4	-	17. 9 ± 0.4
Pseudomonas aeruginosa	10.3 ± 1.1	8.2 ± 0.8	-	11. 0 ± 0.2

Key: (-)=no activity

Table 2. Antimicrobial activities of *Momordica charantia* extract prepared at 200 mg/ml

Diameter of zones of inhibition in mm				
Test Organisms	**Ethanol Extract**	**n hexane extract**	**Water**	**Chloramphenicol**
Staphylococcus aureus	11.2 ± 0.2	9.6 ± 0.4	-	13.3 ± 0.1
Bacillus subtilis	14.9 ± 0.8	12.6 ± 0.7	-	11. 3 ± 1.2
Escherichia coli	17.1 ± 0.2	14.5 ± 0.9	-	18. 0 ± 0.2
Pseudomonas aureginosa	11.9 ± 0.6	9.4 ± 0.2	-	11. 2 ± 0.1

Key (-)=no activity

Table 3. Minimum inhibitory concentration of the leaf extract of *Momordica charantia*

Bacteria	**Concentration (mg/ml)**							
	Ethanol				**n hexane**			
	10.0	**20.0**	**30.0**	**40.0**	**10.0**	**20.0**	**30.0**	**40.0**
S. aureus	+	+	-	-	+	+	-	-
Bacillus subtilis	+	+	-	-	+	+	-	-
Escherichia coli	+	+	-	-	+	+	-	-
Pseudomonas aeruginosa	+	+	+	-	+	+	-	-

KEY: (-) = no growth, (+) = growth

Table 4. Minimum bacteriocidal concentration of the leaf extract of *Momordica charantia*

Concentration (mg/ml)				
Bacteria	**Ethanol**		**n hexane**	
	30.0	**40.0**	**30.0**	**40.0**
S. aureus	+	-	+	-
Bacillus subtilis	-	-	-	-
Escherichia coli	-	-	-	-
Pseudomonas aeruginosa	+	-	+	-

Key: = no growth + = growth

Table 5. Result of phytochemical screening of *Momordica charantia* leaf extract

Test	**Ethanol**	**n hexane**
Tannin	+	+
Saponnin	+	+
Flavonoid	–	–
Alkaloid	+	+
Glycoside	–	–
Anthraquinones	–	+
Polyphenol	+	+

KEY (-) Absent, (+) Present

3.4 Minimum Bacterioocidal Concentration of the Leaf Extract of Momordica *charantia*

The minimum bacteriocidal concentration values of both ethanol and n Hexane ranged from 30 – 40 mg/ml

3.5 Phytochemical Screening of *Momordica charantia* Leaf Extract

The phytochemical screeening of ethanolic extract of *Momordica charantia* leaf indicate the presence of saponins, flavonoid, tannins, alkaloids, anthraquinine glycosides, and polyphenol.

4. DISCUSSION

The antibacterial activities of *Momordica charantia* leaf extract demonstrated that both ethanol and n hexane extracts had varied degrees of antibacterial activity, with ethanol extract having the maximum antimicrobial activity against the test species. This indicates that the solvent

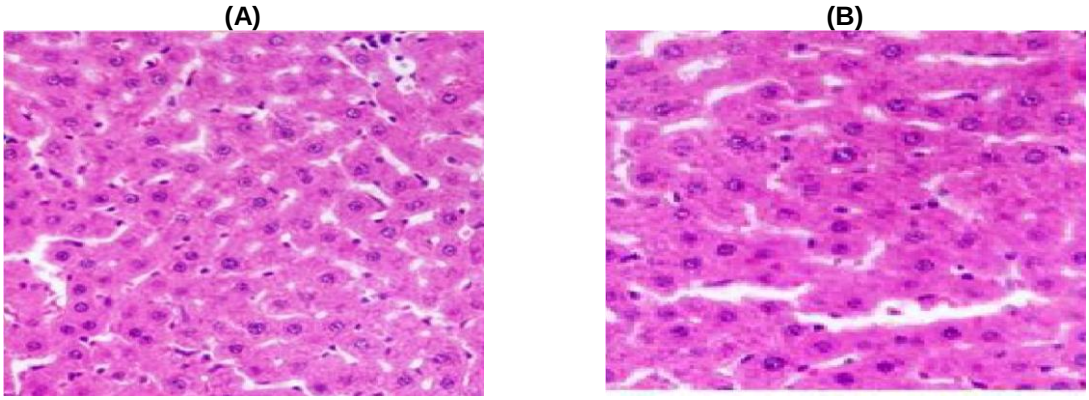

Plate 1. Photomicrograph of histological examination of the liver of rat fed with leaf extracts of *Momordica charantia* *(a)* control rat (b) treated rat both showed normal histological structure of central vein and surrounding hepatocytes

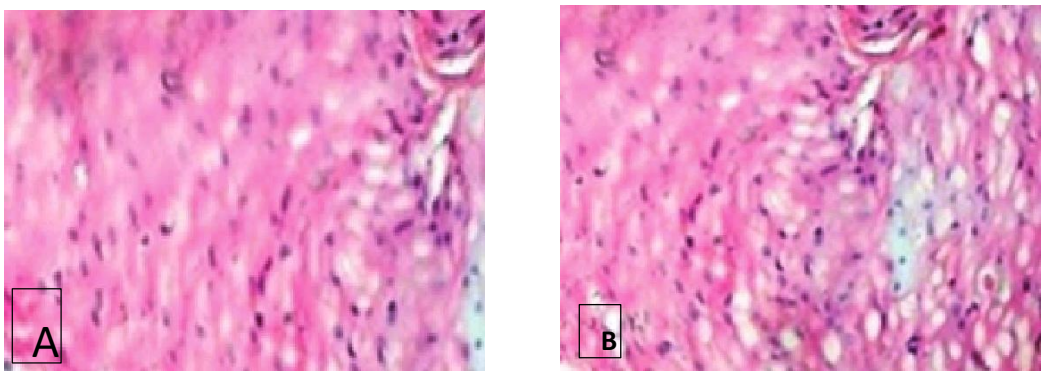

Plate 2. Photomicrograph of histological examination of large intestine of rat fed with leaf extracts of *Momordica charantia* (a) control rat (b) treated rat both showed regular shaped villi lined by intact and moderately crowned columnar epithelium

solution has a significant impact on the bioactive component's solubility and antibacterial activity. However, as compared to typical drugs, the zone of inhibition for ethanol was minimal (Chloramphenicol). When ethanol and n-hexane extract were evaluated, chloramphenicol showed the most antibacterial action. Because chloramphenicol is a conventional antibiotic and is in its purest form, it has the highest action. This research supports the findings of [12] who found that leaf extracts of *Momordica charantia* have broad range antibacterial activity. In both ethanol and n-hexane extracts of *Momordica charantia* leaves, phytochemical screening revealed the presence of Saponins, Tannin, Alkaloid, and Polyphenol, which coincided with previous research [10]. *Momordica charantia*'s antibacterial effects are aided by these substances, which are known to be physiologically active. Antibacterial action is elicited by these secondary metabolites via a variety of ways. Tannin, for example, has been discovered to form irreversible complexes with proline-rich proteins [13] that impede cell protein production [14]. Tannins are known to react with protein to generate the tanning effect, which is useful for treating inflammatory or ulcerated tissues. Tannins are utilized to alleviate illnesses including diarrhea, according to Dharmananda [15]. As a result, our findings support the use of *Momordica charantia* leaves to treat some of the diseases induced by the test organisms. Alkaloids were also found in the leaves of *Momordica charantia,* which are harmful to foreign organisms' cells. These activities have been extensively researched for their potential to eliminate and reduce human cancer cell lines. The major vein and surrounding hepatocytes of the apparently healthy albino rats administered the leaf extract had normal histological structure, and their large intestines had regular shaped villi surrounded with intact, modestly capped columnar epithelium. This showed that the leaf extract is not harmful to internal organs and that

it may be used safely for medical purposes. The findings of antibacterial activity and phytochemical screening of *Momordica charantia* leaf extracts against test organisms back up herbalists' and local populations' assertions that *Momordica charantia* can be used to heal a variety of ailments.

5. CONCLUSION

The leaf extract of *Momordica charantia* demonstrated antibacterial efficacy against Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Bacillus subtilis*. The ethanolic leaf extract had a higher zone of inhibition than the n-hexane extract in this investigation. In addition, the plant showed low minimum inhibitory concentration (MIC) values for both ethanol and methanol, which are critical for determining antimicrobial efficacy. As a result, the plant could be used as a source for developing effective antibacterial agents against the microorganisms studied. The antibacterial activity of Momordica charantia leaf may be due to alkaloid, tannin, and saponin, which were found in this study. As a result, the extract can be used as an antibacterial treatment to treat infections and diseases caused by these bacteria.

6. RECOMMENDATION

Further pharmacological and clinical studies should be done to understand the mechanism of action and the efficacy of the *Momordica charantia* in treating infection and diseases caused by these bacteria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. Clininical Microbiology Review. 1999;12:564–582.
2. Rossiter SE, Fletcher MH, Wuest WM. Natural products as platforms to overcome antibiotic resistance. Chememical Reviews. 2017;117(2):12415–12474.
   DOI: 10.1021/acs.chemrev.7b00283
3. Batran, SAES, El-Gengaihi SE, El-Shabrawya OA. Some toxicological studies of Momordica charantia L. on albino rats in normal and alloxan diabetic rats. Journal of Ethnopharmacology. 2006;108:236-242.
4. Kirtikar KR, Basu BD. In: E. Blatter JF, Caius KS. Mhaskar (2ndEds.), Indian medicinal plants (Vol. 2). Allahabad, India: Lalit Mohan Basu. 1993;1182
5. Sanchez LO, Gustot T. Multidrug-resistant bacterial infection in patients with cirrhosis. A review. Curr Hepatol Rep. 2019;18 (1):28-35.
6. Mbuni YM, Wang S, Mwangi BN, Mbari NJ, Musili PM, et al. Medicinal Plants and Their Traditional Uses in Local Communities around Cherangani Hills, Western Kenya. Plants. 2020;9(331):1-16.
7. Nadkarni KM. In: Indian Materia Medica. Popular Prakashan, Mumbai. 2007;II :296.
8. Satyavati GV, Raina MK, Sharma M. Medicinal Plants of India. ICMR, New Delhi, India. 1987; I:317-320.
9. Gurbuz I, Akyuz C, Yesilada E, Bilge S. Anti-ulcerogenic effect of Momordica charantia L. fruits on various ulcer models in rats. Journal of Ethnopharmacology. 2000;71(2):77-82.
10. Akinpelu DA, Kolawole DO. Phytochemical and antimicrobial activity of leaf extract of Piliostigma thonningii (Schum.). Science Focus Journal. 2004;7(4):64-70.
11. Sankaranarayanan J, Jolly CI. Phytochemical, antibacterial and pharmacological investigations on Momordica charantia Linn.,Emblica offidnalis gaertn and Curcuma longa linn. Industrial. Journal of Pharmacology Science. 1993;55:6-13.
12. Shimada T. Salivary proteins as a defense against dietary tannins. Journal of Chemical Ecology. 2006;32(6):1149 -1163.
13. Parekh J, Chanda S. In vitro antibacterial activity of crude methanol extract of Woodfordia fruticosa Kurz flower (Lythacease).Brazil. Journal of Microbiology. 2007;38(3):2.
14. Dharmananda S. Gallnuts and the uses of Tannins in ChineseMedicine. In:

Proceedings of Institute for Traditional Medicine,Portland, Oregon; 2003.

15. CLSI. M100-S23," in Performance Standards for Antimicrobial Testing; 23rd Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

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