Article

Unveiling the Bioactive Phytochemicals of *Momordica charantia* Leaves and Their Antibacterial Effects

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Abstract: *Momordica charantia* is widely cultivated in Zaria, Nigeria, and holds a prominent place in traditional medicine. Its leaves, fruits, and seeds are known to be rich in bioactive compounds and are commonly employed to treat various infections and diseases. This study aimed to investigate the bioactive components and antibacterial properties of methanolic leaf extracts and their fractions. Phytochemical analysis of the methanolic extract revealed the presence of alkaloids, saponins, flavonoids, glycosides, tannins, steroids, and terpenoids. The methanolic extract was fractionated into n-butanol, ethyl acetate, and chloroform fractions. The methanolic extract exhibited superior antibacterial activity compared to its fractions, suggesting potential synergistic effects among the plant's constituents. Antibacterial efficacy was evaluated using well-diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. *Salmonella typhi* was the most susceptible bacterium, with zones of inhibition of 25.00 ± 0.10 mm, MIC of 12.5 ± 0.82 mg/mL, and MBC of 50 ± 0.22 mg/mL. This was followed by *Escherichia coli* (18.77 ± 0.25 mm, MIC: 50 ± 0.53 mg/mL, MBC: 100 ± 0.82 mg/mL) and *Staphylococcus aureus* (14.13 ± 0.91 mm, MIC: 50 ± 0.23 mg/mL, MBC: 100 ± 0.48 mg/mL). Among the fractions, the n-butanol fraction demonstrated the highest antibacterial activity. Subsequent analysis of this fraction using GC-MS identified key compounds, including 2-pentanone, 4-hydroxy-4-methyl-, n-amyl isovalerate, 2(5*H*)-furanone, 3,5,5-trimethyl-, furan, tetrahydro-2,2,4,4-tetramethyl-, and 3-tetradecanol acetate. In conclusion, the methanolic extract followed by n-butanol fraction of *M. charantia* exhibited significant antibacterial activity, particularly against Gram-negative bacteria such as *S. typhi* and *E. coli*. Further research is recommended to isolate and characterize the bioactive compounds responsible for this activity.

Keywords: secondary metabolites; bacteria, natural product; medicinal plants; GC-MS analysis

1. Introduction

For millennia, *Homo sapiens* have recognized the therapeutic benefits of certain plants in treating various illnesses. Fossil records from Iraq, dating back 60,000 years, provide evidence of this practice. Nonetheless, the use of medicinal herbs likely extends even further, given that anatomically modern humans emerged in Africa around 300,000 years ago [1], and throughout ancient civilizations herbs are being used in the treatment of diseases and revitalize the body system [2]. Plants are recognized not only for their nutritional benefits but also for providing remedies. As such they are recognized for their medicinal and healing properties, which are often commonly referred to as medicinal plants [3]. Medicinal plants are characterized by the presence of bioactive metabolites that can be utilized for medicinal purposes for synthesis of drugs or medicinal agent [4]. Several herbs frequently used in traditional treatments include *Picrorhiza* sp., garlic, cloves, neem (both fruit and leaves), nutmeg, cinnamon, ginger, peppermint, sage, thyme, mustard, and fenugreek [5,6]. These plants serve various purposes such as promoting digestion, alleviating diarrhoea, acting as antiseptics and anti-inflammatories, combating parasites, and enhancing appetite in both humans and animals [3].

Thousands of compounds, including phytochemicals and other bioactive polyphenolic compounds, have been isolated from various varieties of medicinal plants and showed potent bioactive properties [7]. The phytochemicals isolated in various plants include phenolic acids, tannins, flavonoids, saponins, cardiac glycosides, anthocyanins, anthraquinones, and terpenoids [4]. Some of them, like tannins, flavonoids and saponins were reported to possess good antimicrobial activity [8]. However, microbes, including bacteria, have developed resistance against antimicrobial agents, leading to emerging multi-drug resistant bacteria. The growing ineffectiveness of

chemotherapeutics and the rise in antibiotic resistance of pathogenic microbes have spurred the screening of various medicinal plants for their potential antimicrobial activities [9]. Despite pharmaceutical breakthroughs in producing new antibiotics, bacterial resistance persists leading to ongoing development of resistance to current antibacterial drugs which underscores the need for exploring new antimicrobial agents [10].

Nanotechnology is a rapidly advancing field with broad applications across science and technology, particularly in the synthesis and development of nanomaterials and nanoparticles. nanoparticle technology has demonstrated the ability to overcome bacterial drug resistance mechanisms by inhibiting biofilm formation and disrupting other key processes linked to bacterial virulence [11]. Green-synthesized silver nanoparticles (AgNPs) have demonstrated promising antibacterial activity against a wide spectrum of Gram-positive and Gram-negative pathogenic bacteria. These include *Salmonella* spp., *Pseudomonas* spp., *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Bacillus* spp., *Vibrio* spp., and many more [12]. *S. typhimurium*, among others, are the most common agents causing bacterial diseases, especially associated with unhygienic practices [13]. These species are known to develop strains that are resistant to available antibiotics. Due to challenges in antibacterial chemotherapy, our goal is to address multi-drug resistance through testing clinical bacterial isolates by leveraging medicinal plants as the primary source of natural antibacterial agents. A previous finding on the study of efficacy of *Moringa oliefera* leaves extract showed significant antibacterial activity against *S. aureus* and *E. coli* [14]. The further suggest that the efficacy of the plant's extract against *Shigella* sp. is concentration dependent. In literature, Adamu et al. [15], have documented the antibacterial potential of *Citrus sinensis* leaf extract against *S. typhi* and *S. paratyphi* and showed the potentiality of citrus leaves extract in management of Salmonella's infections. Bashir et al. [16], have also reported the effectiveness of Baobab leaves and stem bark extract against the growth of clinical bacterial isolates (*S. typhi, S. aureus* and *E. coli*) with more activity recorded in leaf extract against these bacteria.

Little is known about the biological properties of *Momordica charantia* grown in Nigeria. *Momordica charantia* (*M. charantia*) common name is Bitter melon, is belonging to the Cucurbitaceae family, is renowned for its bitter taste. The unripe fruit is believed to contribute to optimal health. The plant, particularly its fruits, has shown numerous indications of therapeutic properties [17]. The plant is grown in tropical and subtropical regions, including India, Asia, South America, and Nigeria. In various parts of the world, including South American countries. *M. charantia* is extensively cultivated for both culinary and medicinal purposes because of its properties such as hypoglycemic activity, anti-HIV, antitumor, antidiabetic, antileukemic, anticancer, anti-inflammatory, antioxidant; In African countries it has been used for its antimalarial property [18,19]. A previous study by Hassan et al. [20], have reported antifungal efficacy of methanolic extract of *M. charantia* fruit. Another study conducted on the anticancer effect of *M. charantia* fruits documented the potentiality of the methanolic extract in the inhibition of cancer cell line growth [21]. Similarly, Zeyp et al. [22], cited anti-hyperglycemic effects of *M. charantia* fruits. Gultom et al. [23], reported a findings that have demonstrated strong antibacterial potential of the ethanolic leaf extract of *M. charantia*. However, the authors did not record much activities against *S. aureus* and *S. typhi* which may likely be due to the type of solvents used in the extraction process. Despite researchers' efforts to harness the medicinal attributes of *M. charantia* leaves. In general, little is known about its antibacterial properties, especially when utilizing different solvents and fractions in the extraction. Therefore, the current study aimed to evaluate the antibacterial potentials of methanolic extract and three fractions of *M. charantia* leaves grown in Nigeria against some bacterial pathogens, beside, unveiling its phytochemical contents.

2. Materials and Methods

2.1. Plant Samples

The *M. charantia* leaves were collected from the Zaria Local Government Area in Kaduna State, located at coordinates 11°04′ N and 7°42′ E. Collection was conducted during the dry season, with a temperature of 38 °C. The leaves were sent to the laboratory in sanitized polythene bags. A taxonomist from the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria, verified the plant, awarding them voucher number 1697. The fresh leaves were washed with distilled water, air-dried in the shade at ambient temperature, then crushed into a fine powder using a mortar and pestle. The powdered samples were preserved in sterile polythene bags for further examination.

2.2. Preparation of Extract

The methanolic extract of the plant leaves was prepared following the method described by Yusuf et al. [24]. A total of 100 g of plant material was suspended in 500 mL of absolute methanol in conical flasks and vigorously shaken for a few minutes. The mixture was left to stand for 72 h, then filtered first with muslin cloth and

subsequently with filter paper. The filtrate was concentrated in a water bath at 40 °C for 48 h. The resulting dry crude extract was stored in a sterile container and kept in a refrigerator at 4 °C until needed for use.

2.3. Qualitative Phytochemical Screening

The methanolic extract of *M. charantia* leaves was screened for the presence of secondary metabolites following the methods outlined by Trease and Evans [25]. The metabolites tested for included carbohydrates, flavonoids, saponins, tannins, terpenoids, steroids, anthraquinones, alkaloids, and cardiac glycosides:

2.3.1. Test for Carbohydrates

Molisch's test was used to assess carbohydrate content. Three drops of Molisch's reagent were added to the methanolic leaf extract in a test tube, followed by concentrated sulfuric acid. The formation of a reddish ring at the interface indicated the presence of carbohydrates.

2.3.2. Test for Saponins

Two milliliters of the extract were mixed with 10 mL of distilled water and shaken vigorously for 30 s. The formation of a persistent 2 cm layer of foam after 5 min indicated the presence of saponins.

2.3.3. Test for Flavonoids

To test for flavonoids, 1 mL of NaOH was added to 3 mL of the extract. The appearance of a yellow color confirmed the presence of flavonoids.

2.3.4. Test for Tannins

The presence of tannins was tested by adding 3 drops of 0.1% ferric chloride to 2 mL of the extract. A brownish-green precipitate confirmed the presence of tannins.

2.3.5. Test for Terpenoids

Two milliliters of chloroform were added to 5 mL of the extract, followed by 3 mL of concentrated sulfuric acid. A reddish coloration at the interface indicated the presence of terpenoids.

2.3.6. Test for Alkaloids

Wagner's reagent was added to 2 mL of the extract. The formation of an orange-brown precipitate indicated the presence of alkaloids.

2.3.7. Test for Anthraquinones

The sample was dissolved in chloroform, filtered, and shaken with 10% ammonia solution. The appearance of a bright pink color in the upper aqueous layer indicated the presence of anthraquinones.

2.3.8. Test for Cardiac Glycosides

A portion of the extract was dissolved in glacial acetic acid containing traces of ferric chloride, and 1 mL of sulfuric acid was carefully added. The presence of a brown ring at the interface indicated the presence of cardiac glycosides.

2.4. Identification of Bactreia

Standard experimental procedures were employed to identify the bacterial strains. A full loop of the stock cultures of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* was streaked onto both blood agar and nutrient agar plates, followed by incubation for 18–24 h at 37 °C. Colony morphology was observed and documented, after which individual colonies were selected for further confirmation tests. These tests included Gram staining and culture on various differential media such as MacConkey agar, xylose lysine deoxycholate agar, Salmonella-Shigella agar, mannitol salt agar, and eosin methylene blue agar. In addition, biochemical identification assays, including the indole test, oxidase test, coagulase test, catalase test, motility test, Simmon's citrate test, MRVP (methyl-red, Voges-Proskauer) test, and TSI (triple sugar iron) test, were conducted following established protocols [26].

2.5. Antibacterial Activity

The antibacterial activity of the plant extract was evaluated using the agar well diffusion method. A standardized bacterial suspension was used to prepare an inoculum, of which 0.1 mL was applied onto Mueller-Hinton agar plates, with each test conducted in triplicate. The inoculum was uniformly spread across the plate surface using a sterile cotton swab. After allowing the plates to rest for 10 min, wells with a 6 mm diameter were punched into the agar using a sterile cork borer. Each well was filled with 0.1 mL of the plant extract at varying concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL). Wells containing dimethyl sulfoxide (DMSO) were used as negative controls. The plates were left at room temperature for 10 min to enable diffusion of the extract into the agar, followed by incubation at 37 °C for 24 h. After incubation, inhibition zones were observed, and the diameters of these zones were measured in millimeters using a ruler. The means and standard deviations were then statistically calculated [27].

2.6. MIC and MBC Assays

To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts against the tested bacteria were determined following a modified version of previously published methods [28]. In summary, 1 mL of Mueller-Hinton broth was dispensed into five sterilized test tubes. A 50% extract concentration was prepared by adding 1 mL of the plant extract to the first test tube, followed by a series of twofold dilutions to create concentrations of 50%, 25%, 12.5%, 6.25%, and 3.12%. Subsequently, 100 µL of a standardized bacterial culture was added to each tube. The lowest concentration that exhibited no turbidity, indicating inhibition of bacterial growth, was recorded as the MIC. For the MBC determination, 50 µL from each tube showing no turbidity was plated on nutrient agar and incubated at 30 °C to 35 °C overnight. The lowest concentration at which no bacterial growth was observed on the agar plates was identified as the MBC.

2.7. Fractionation of the Extract

The most active components of the extract were fractionated using different solvents: chloroform, ethyl acetate, and n-butanol. *M. charantia* methanol extract was subjected to fractionation to separate its active components. The process involved dissolving approximately 100 g of the crude extract in 1 litre of distilled water using a separator funnel. Then, 250 mL of chloroform was mixed with 250 mL of the extract solution (1:1 *v*/*v*). The funnel was vigorously shaken, and after allowing it to settle for 15 min, the organic solvent layer was separated from the aqueous layer. The chloroform layer was isolated and collected in a clean flask by carefully opening the funnel knob. This separation process was repeated sequentially with ethyl acetate and n-butanol. The collected soluble fractions were labelled and concentrated to dryness under vacuum using a rotary evaporator. Each extract from the soluble fractions was securely sealed in a clean container and stored in a refrigerator at 4 °C until further use [26].

2.8. GC-MS Analysis

The extract was dissolved in ethanol along with a mixture of solvents, including toluene, chloroform, ethanol, and ethyl acetate, before being subjected to GC-MS analysis. The analysis was performed at Kaduna State University, 800283, Kaduna State, Nigeria. Helium (99.999%) served as the carrier gas, with a flow rate of 1 mL/min. The separation was carried out using an HP5 column with a length of 30 mm, an internal diameter of 0.32 mm, a film thickness of 0.25 mm, and a temperature range from −60 °C to 325 °C (maximum 350 °C). The total GC run time was 35 min, with the oven temperature increasing from 70 °C to 280 °C at a rate of 8 °C per minute. A sample volume of 4 µL was injected, and the MS was operated at 70 eV. Compound identification was based on comparing the spectra of unknown compounds with those in the reference library, allowing for probable determination of their names, molecular weights, and structures [29].

2.9. Statistical Analysis

The agar well diffusion assay was performed in triplicate, and the results were reported as means ± standard deviation (SD). Analysis of Variance (ANOVA) was utilized to evaluate significant differences among the isolates compared to the control. A p-value of < 0.05 was considered statistically significant for assessing the observed differences.

3. Results and Discussion

The Percentage

Phytochemical analysis of the methanolic leaf extract of *M. charantia* (Table 1) demonstrated the presence of steroids, alkaloids, saponins, flavonoids, carbohydrates, cardiac glycosides, tannins, and terpenoids, while anthraquinones were notably absent. This study corroborates previous findings regarding the presence of saponins, flavonoids, and alkaloids [14,24]. Alkaloids exhibit antibacterial properties by interfering with the peptidoglycan structure in bacterial cells, inhibiting cell wall synthesis and leading to cell death. Additionally, alkaloids function as DNA intercalators, hindering the activity of bacterial cell topoisomerase enzymes [21,30]. The qualitative analysis of the methanolic extract also indicated the presence of phenolic compounds. Valizadeh et al. [31] and Rahmi and Sari [32] similarly reported the presence of flavonoids, saponins, and phenols in the ethanolic extracts of *M. charantia* fruits and leaves.

Table 1. Phytochemical Constituents of *M. charantia* methanolic leaf extract.

Bioactive Components	*M. charantia*
Alkaloids	+
Saponins	+
Flavonoids	+
Steroids	+
Terpenes	+
Cardiac glycosides	+
Anthraquinones	−
Tannins	+
Carbohydrates	+

KEY:+: Presence; − : Absence.

Saponins act as antibacterial agents by reducing surface tension, enhancing permeability, and promoting cell leakage. This increased permeability allows for the diffusion of intercellular substances through the cytoplasmic membrane, compromising cell membrane integrity and resulting in cytoplasmic leakage and cell death [33]. The discrepancies observed in these investigations may be attributed to the solvents utilized in Soxhlet extraction, as the choice of solvent and plant component yield varying results during preliminary phytochemical screenings. Notably, saponins exhibit anti-inflammatory properties, hemolytic activity, and cholesterol-binding capabilities, while flavonoids mitigate cellular oxidative stress and demonstrate beneficial effects as anticancer and antibacterial agents [34].

Table 2 presents the mean inhibition zones of *M. charantia* against Staphylococcus aureus, Salmonella typhi, and Escherichia coli at varying concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL. The results indicate that the antibacterial activity of *M. charantia* extract is concentration-dependent, with the highest mean inhibition zone observed against *S. typhi* (25.0 mm), followed by *E. coli* (18.77 mm) and *S. aureus* (14.13 mm). These findings are consistent with recent research by Lawrence and Olusola [35], which also demonstrated significant antibacterial activity against *S. aureus* and *E. coli*. Notably, high inhibition zones were recorded for *S. typhi* and *E. coli*. The observed variations in inhibition may come from the structural differences in bacterial membranes; *S. aureus*, as a Gram-positive organism, possesses an extracellular envelope that can confer resistance to certain antibacterial agents. The differing susceptibility among these bacteria may be attributed to the permeability barriers presented by the lipopolysaccharide-rich outer membranes of Gram-negative bacteria [36]. Another study found that *Momordica charantia* exhibited no inhibitory effects against the tested bacterial isolates, including *E. coli, Klebsiella pneumoniae*, *Proteus mirabilis,* and *S. typhi* [37]. The notable inhibition of *S. typhi* is likely related to the presence of alkaloids and flavonoids in *M. charantia*, which have been documented to inhibit cellular membrane synthesis and act as bacterial cytotoxins [19]. In contrast, Hassan et al. [20] reported no inhibition zone for *S. typhi* at concentrations of 25 mg/mL and 50 mg/mL of *M. charantia* fruit extract. The discrepancies in these studies suggest that *M. charantia* fruit may possess lower concentrations of phytonutrients compared to its leaves, despite the same solvent being used for extraction.

Table 2. Antibacterial activity of *M. charantia* methanolic leaf extract against bacterial isolates.

	Mean Zones of Inhibition (mm) ± Standard Deviation of *M. charantia*				
	Concentration (mg/mL)				
Bacterial	**100**	**50**	**25**	**12.5**	**Ciprofloxacin**
Staphylococcus aureus	14.13 ± 0.91 [c]	11.83 ± 0.31 [c]	5.90 ± 0.10 [c]	3.03 ± 0.15 [c]	35.43 ± 0.31 [c]
Salmonella typhi	25.00 ± 0.10 [a]	20.43 ± 0.12 [a]	13.97 ± 0.41 [a]	7.76 ± 0.46 [a]	39.13 ± 0.47 [b]
Escherichia coli	18.77 ± 0.25 [b]	16.23 ± 0.25 [b]	12.70 ± 0.44 [b]	6.90 ± 0.10 [b]	42.47 ± 0.15 [a]

Results are means ± standard deviation of triplicate (n = 3) determinations. Values with different superscripts in the column differ significantly at $p < 0.05$ using the Duncan Multiple Range Test.

The MIC and the MBC results of the methanolic leaf extracts of *M. charantia* against the bacterial isolates are summarized in Table 3. The MIC values recorded were 50 mg/mL for *S. aureus*, 12.5 mg/mL for *S. typhi*, and 50 mg/mL for *E. coli*, while the MBC values were 100 mg/mL for *S. aureus*, 50 mg/mL for *S. typhi*, and 100 mg/mL for *E. coli*. The lowest zone of inhibition was noted for *S. typhi* at 12.5 mg/mL compared to the other bacterial species. The reduced inhibition observed for *S. typhi* may be due to characteristics inherent to its bacterial cell membrane. The MIC results for *S. typhi* are in agreement with recent findings by Shahrajabian et al. [38] (2023), who also reported high efficacy of *M. charantia* fruit extract against this pathogen. However, our study demonstrated significant inhibition at a lower concentration of 12.5 mg/mL compared to the 100 mg/mL reported previously. Furthermore, a study on *M. charantia* seed extract showed effective inhibition against *S. aureus* at concentrations as low as 50 mg/mL [8]. Despite the same solvent being used for extraction, our results indicated lower inhibition zones, suggesting that the leaves of *M. charantia* may contain higher concentrations of phytonutrients capable of eliciting stronger antibacterial effects compared to seeds and fruits. Sherekar et al. [18] recommend a concentration of 100 mg/mL as sufficient for exerting antibacterial activity against various bacterial strains, indicating that extract of *M. charantia* can demonstrate antibacterial effects against both Gram-negative and Gram-positive bacteria, contingent upon the solvent and extraction method utilized.

Table 3. Minimum Inhibitory Concentration and Minimum Bacterial Concentration of *M. charantia* leaf extract.

Bacteria	**MIC (mg/mL)**	**MBC (mg/mL)**
S. aureus	50 ± 0.23 [a]	100 ± 0.48 [b]
S. typhi	12.5 ± 0.82 [c]	50 ± 0.22 [a]
E. coli	50 ± 0.53 [a]	100 ± 0.82 [b]

Results are means ± standard deviation of triplicate (n = 3) determinations. Values with different superscripts in the column differ significantly at $p < 0.05$ using the Duncan Multiple Range Test (DMRT).

The results of antibacterial tests for the n-butanol, ethyl acetate, and chloroform fractions of *M. charantia* against *S. aureus*, *S. typhi*, and *E. coli* (Tables 4–7) were evaluated at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, respectively. The results revealed that the antibacterial activity of the extract is concentration-dependent, which agrees with previous studies on various medicinal plants tested [39,40]. The highest mean inhibition observed against *S. typhi* in both n-butanol and ethyl acetate fractions, while no significant difference ($p < 0.05$) was noted in the chloroform fraction. Both fractions exhibited the lowest zone of inhibition for *S. typhi*. Similar findings by Singh et al. [41] also reported reduced zones of inhibition for *S. typhi*. Previous studies indicate that anthraquinones possess intercalating activity against plasma phospholipids, inducing oxidative stress and increasing the likelihood of bacterial cell rupture [33]. This suggests that chloroform may not be a good solvent for extracting antibacterial compounds from *M. charantia*.

Table 4. Antibacterial Activity of n-butanol fraction of *M. charantia.*

	Mean Zones of Inhibition (mm) ± Standard Deviation of *M. charantia*				
	Concentration (mg/mL)				
Bacterial	**100**	**50**	**25**	**12.5**	**Ciprofloxacin**
Staphylococcus aureus	10.67 ± 0.15 [b]	7.50 ± 0.10 [c]	4.67 ± 0.21 [c]	3.33 ± 0.15 [c]	35.50 ± 0.50 [c]
Salmonella typhi	12.63 ± 0.15 [a]	11.10 ± 0.10 [a]	9.63 ± 0.12 [a]	6.10 ± 0.10 [a]	38.80 ± 0.44 [b]
Escherichia coli	9.87 ± 0.25 [c]	8.40 ± 0.10 [b]	6.40 ± 0.10 [b]	4.33 ± 0.06 [b]	42.63 ± 0.15 [a]

Values are means ± standard deviation of triplicate (n = 3) determinations. Values with different superscripts in the column differ significantly at $p < 0.05$ using the Duncan Multiple Range Test (DMRT).

Table 5. Anti-bacterial Activity of ethyl acetate fraction of *M. charantia* against bacterial isolates.

	Mean Zones of Inhibition (mm) ± Standard Deviation of *M. charantia*				
	Concentration (mg/mL)				
Bacterial	**100**	**50**	**25**	**12.5**	**Ciprofloxacin**
Staphylococcus aureus	7.43 ± 0.05 [b]	4.60 ± 0.10 [b]	3.53 ± 0.06 [b]	2.23 ± 0.15 [b]	35.5 ± 0.50 [c]
Salmonella typhi	8.07 ± 0.06 [a]	6.23 ± 0.21 [a]	5.20 ± 0.26 [a]	3.23 ± 0.06 [a]	38.80 ± 0.44 [b]
Escherichia coli	6.60 ± 0.10 [c]	4.43 ± 0.06 [b]	2.93 ± 0.06 [c]	1.53 ± 0.06 [c]	42.63 ± 0.15 [a]

Values are means ± standard deviation of triplicate (n = 3) determinations. Values with different superscripts in the column differ significantly at $p < 0.05$ using the Duncan Multiple Range Test (DMRT).

Table 6. Anti-bacterial activity of chloroform fraction of *M. charantia* against bacterial isolates.

	Mean Zones of Inhibition (mm) ± Standard Deviation of *M. charantia*				
	Concentration (mg/mL)				
Bacterial	**100**	**50**	**25**	**12.5**	**Ciprofloxacin**
Staphylococcus aureus	5.13 ± 0.06 [a]	3.20 ± 0.10 [b]	2.36 ± 0.15 [b]	1.27 ± 0.06 [c]	35.50 ± 0.50 [c]
Salmonella typhi	5.10 ± 0.10 [a]	4.20 ± 0.10 [a]	2.57 ± 0.06 [a]	1.90 ± 0.10 [a]	38.80 ± 0.44 [b]
Escherichia coli	5.10 ± 0.10 [a]	4.03 ± 0.12 [a]	2.27 ± 0.06 [b]	1.53 ± 0.15 [b]	42.63 ± 0.15 [a]

Values are means ± standard deviation of triplicate (n = 3) determinations. Values with different superscripts in the column differ significantly at $p < 0.05$ using the Duncan Multiple Range Test (DMRT).

Table 7. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of fractions of *M. charantia* against bacterial isolates.

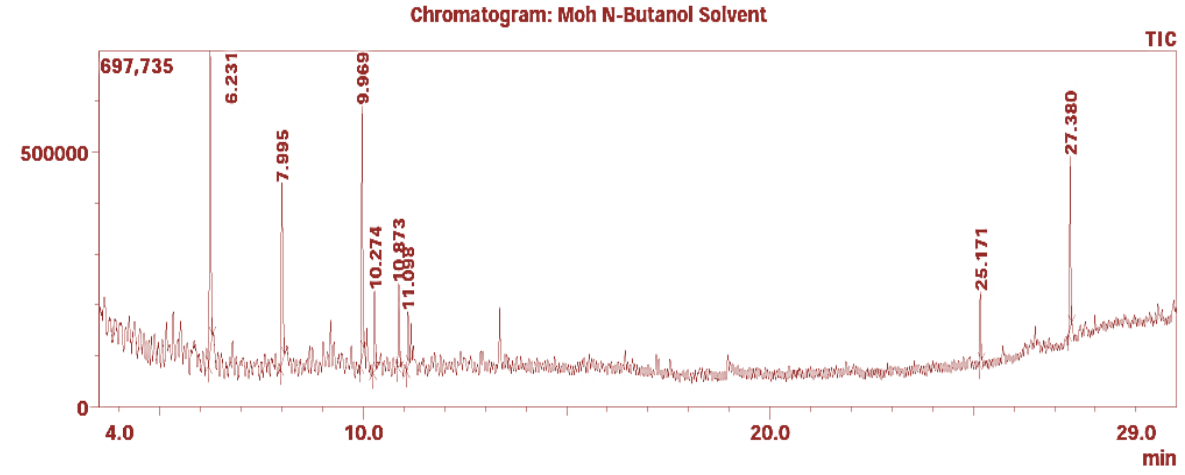
Bacteria	**MIC (mg/mL)**			**MBC(mg/mL)**		
	N-Butanol	**Ethyl Acetate**	**Chloroform**	**N-Butanol**	**Ethyl Acetate**	**Chloroform**
S. aureus	50 ± 0.61 [a]	100 ± 0.34 [a]	100 ± 0.28 [a]	100 ± 0.61 [a]	-	-
S. typhi	25 ± 0.32 [b]	50 ± 0.12 [b]	50 ± 0.31 [b]	50 ± 0.14 [b]	100 ± 0.28 [c]	100 ± 0.88 [b]
E. coli	50 ± 0.42 [a]	100 ± 0.88 [a]	100 ± 0.18 [a]	100 ± 0.89 [a]	-	-

Values are means ± standard deviation of triplicate (n = 3) determinations. Values with different superscripts in the column differ significantly at $p < 0.05$ using the Duncan Multiple Range Test (DMRT).

The MIC and MBC assays are critical for evaluating the inhibitory effects of plant extracts [42], and sometimes MBC/MIC ratio is calculating to understand the mode of action [43]. Our findings indicate that the n-butanol fraction of M. charantia (Table 4) exhibited the highest antibacterial potency, with the lowest MIC values of 50 mg/mL against *S. aureus*, 25 mg/mL against *S. typhi*, and 50 mg/mL against *E. coli*. This fraction also demonstrated bactericidal activity, as evidenced by the MBC of 100 mg/mL against both *S. aureus* and *S. typhi*. Overall, our assays revealed inhibition of all tested organisms at extract concentrations below 100 mg/mL for the n-butanolic fraction. This MBC result corroborates our preceding MIC findings. A related study by Rahmi and Sari [32] also demonstrated the efficacy of fresh leaf extracts of *M. charantia* against *S. aureus*, while the ethyl acetate extract from the same plant part showed no significant antibacterial effect. Likewise, Validezh et al. [31] reported on the efficacy of *M. charantia* fractions against *E. coli*. The observed differences suggest that the leaves of *M. charantia* may contain higher concentrations of phytonutrients compared to seeds. Previous studies have also confirmed the superior antibacterial efficacy of leaf extracts over fruits and seeds of *M. charantia* [20,22].

The results of GC-MS are presented in Figure 1 and Table 8. The GC-MS analysis of the n-butanol fraction of *Momordica charantia* identified a diverse array of compounds, including 2-pentanone, 4-hydroxy-4-methyl- ($C_6H_{12}O_2$), n-amyl isovalerate ($C_{10}H_{20}O_2$), and squalene ($C_{30}H_{50}$), each exhibiting distinct molecular weights and high similarity indices, indicative of their potential biological activities. The presence of esters and ketones suggests significant antibacterial properties, with compounds such as 2(5*H*)-furanone and di-n-octyl phthalate highlighting the extract's complexity and potential for broader pharmacological applications. Notably, squalene's high similarity index underscores its recognized antioxidant and health-promoting benefits, positioning *M. charantia* as a promising source of natural bioactive agents. These findings warrant further exploration of the synergistic interactions among these compounds and their mechanisms of action in antimicrobial therapies and functional applications. Previous research indicated that GC-MS analysis of the leaves and fruits of *M. charantia* resulted in the identification of 18 compounds, whereas hydrodistillation yielded 21 compounds. Notably, benzaldehyde, linalool, and β-cyclocitral were detected using both techniques, with linalool being the predominant compound in each method reflecting their potential medicinal applications and common use in herbal practices [44]. It was also reported that there is a strong correlation of the GC-MS-based metabolite profile of *Momordica*

charantia fruit and its antioxidant activity [45]. To the best of our knowledge, there is currently no study that links experimentally the correlation between the GC-MS-based metabolite profile of *Momordica charantia* and its antibacterial potential. Therefore, we recommend conducting further studies on this topic. Our study provides preliminary evidence that these metabolites may act either independently or synergistically to contribute to the observed antibacterial activity of *Momordica charantia*.

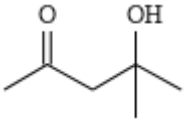
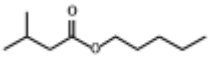
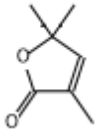
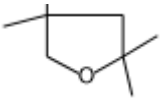
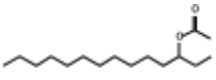
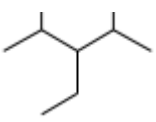


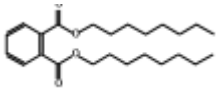
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	6.231	6.190	6.290	1531838	29.63	591280	25.21	2.59	2-Pentanone, 4-hydroxy-4-methyl-
2	7.995	7.960	8.065	982302	19.00	354992	15.13	2.77	
3	9.969	9.925	10.015	947603	18.33	493668	21.05	1.92	2(5H)-Furanone, 3,5,5-trimethyl-
4	10.274	10.240	10.330	369414	7.15	158104	6.74	2.34	Furan, tetrahydro-2,2,4,4-tetramethyl
5	10.873	10.840	10.920	319913	6.19	163303	6.96	1.96	3-Tetradecanol acetate
6	11.098	11.065	11.135	206082	3.99	109235	4.66	1.89	Pentane, 3-ethyl-2,4-dimethyl-
7	25.171	25.140	25.205	214461	4.15	137044	5.84	1.56	Di-n-octyl phthalate
8	27.380	27.340	27.420	598275	11.57	337926	14.41	1.77	Squalene
				5169888	100.00	2345552	100.00		

Figure 1. Gas Chromatography, Mass Spectroscopy analysis of n-butanol fraction of *M. charantia.*

Table 8. Molecular formula and structures of probable compounds identified in the n-butanol fraction of *M. charantia* by GC-MS.

Compound	Formula	Molecular Weight	Similarity Index (SI)	Structure
2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116	87	
n-Amyl isovalerate	$C_{10}H_{20}O_2$	172	89	
2(5*H*)-Furanone, 3,5,5-trimethyl-	$C_7H_{10}O_2$	126	84	
Furan, tetrahydro-2,2,4,4-tetramethyl-	$C_8H_{16}O$	128	81	
3-Tetradecanol acetate	$C_{16}H_{32}O_2$	256	80	
Pentane, 3-ethyl-2,4-dimethyl-	C_9H_{20}	128	81	

Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	86	
Squalene	$C_{30}H_{50}$	410	90	

4. Conclusion

This study highlights the antibacterial potential of *Momordica charantia* methanolic leaf extract and its fractions, particularly the n-butanol fraction, against gram-negative pathogens like *Salmonella typhi* and *Escherichia coli*. The presence of bioactive compounds such as alkaloids, saponins, flavonoids, glycosides, and terpenoids, confirmed through phytochemical screening, supports its use in traditional medicine. Notably, the antibacterial activity observed, especially the good inhibition against *S. typhi* and *E. coli*, underscores the potential of *M. charantia* as a source of antibacterial molecules. However, this preliminary study recommends further advanced techniques such as nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC), and mass spectrometry could be employed to identify and purify these bioactive compounds. Additionally, in vivo studies should be conducted to assess the safety, toxicity, and therapeutic efficacy of the bioactive components of *M. charantia*. Such research would offer valuable insights into the pharmacokinetics and pharmacodynamics of these compounds, guiding their potential clinical application. The development of novel dosage forms, such as nano-formulations or topical applications, could also enhance the delivery and effectiveness of *M. charantia* molecules in controlling bacterial infections.

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