

Article

Evaluation of the Phytochemical Composition and Antibacterial Efficacy of *Momordica balsamina* and *Luffa aegyptiaca* Leaf Extracts

Muhammad Shehu Giwa¹, Basira Ibrahim¹, Fatima Musa¹, and Emad M. Abdallah^{2,3,*}¹ Department of biological sciences, Faculty of Life Sciences, College of Science, Computing and Engineering, Kaduna State University, Kaduna 800283, Nigeria² Department of Biology, College of Science, Qassim University, Qassim 51452, Saudi Arabia³ Faculty of Health and Life Sciences, INTI International University, Nilai 71800, Negeri Sembilan, Malaysia

* Correspondence: emad100sdl@yahoo.com

Received: 21 September 2024; Revised: 7 October 2024; Accepted: 2 November 2024; Published: 17 January 2025

Abstract: The current study evaluated the antibacterial activities of methanol leaf extracts from *Momordica balsamina* and *Luffa aegyptiaca* against clinical isolates of *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. Phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, flavonoids, and tannins, while anthraquinones were absent. Both extracts demonstrated significant antibacterial activity, particularly against Gram-negative bacteria (*S. typhi*), with minimum inhibitory concentrations (MIC) as low as 12.5 mg/mL and minimum bactericidal concentrations (MBC) of 25 mg/mL for *M. balsamina*, and MIC of 12.5 mg/mL and MBC of 50 mg/mL for *L. aegyptiaca*. These findings suggest that these plants have potential as sources of antibacterial agents, warranting further pharmaceutical investigation.

Keywords: antibacterial activity; in vitro study; ethnomedicine; plant extract; bioactive compounds

1. Background

Herbal medicine is now regarded as a rapidly expanding health system globally. Approximately 80% of the global population depends on medicinal plants for primary healthcare, indicating a hopeful future for health maintenance via these natural resources [1]. The use of medicinal plants as remedies predates the emergence of Western medicine alongside the advancement of science and technology. It may serve as a viable alternative source for several medicines, especially after the recent significant failures of antibiotics against pathogens [2]. Recently, it is estimated that there are 14 million deaths due to infections, making them the second most common cause of death after heart diseases. Bacterial pathogens were responsible for 7.7 million of these fatalities, with antibiotic-resistant bacteria contributing to 1.3 million deaths, highlighting the urgent need for novel classes antibiotics [3,4].

Medicinal plants are plants in which one or more of their organs produce substances that can be used for medicinal purposes or as precursors for pharmaceutical synthesis. The practice of herbal medicine traces its origins to the earliest epochs of human history. Historical evidence indicates the utilization of herbs across various ancient civilizations, including Egyptian, Chinese, Greek, and Roman societies, for the treatment of diseases and the restoration of bodily vitality [5,6]. Hence, numerous plants have been acknowledged for their therapeutic and curative attributes and are commonly referred to as medicinal plants. Plants like picorhiza, garlic, cloves, slippery elm, neem fruit and leaves, Kushen, nutmeg, cinnamon, ginger, peppermint, sage, thyme, mustard and fenugreek were studied and reported to possess organic compounds that are vital for human medicine and provide a cure for infection by microbial agents [7].

Phytonutrients are among the chemicals detected to possess pharmacological value in medicinal plants. Phytochemicals exhibit properties that aid in ameliorating bacterial infections by limiting their growth in host cells. Certain phytochemicals, such as alkaloids, flavonoids, tannins, and glycosides, exert their medicinal purpose against microbial infections by limiting bacterial cell wall synthesis, cell division, and DNA repair mechanisms [8]. Saponins aid in the reduction of bacterial proliferation by creating free radicals that hinder bacterial cell growth [9].

Many plants have been screened and tested for their antimicrobial efficacy against certain bacterial strains by studying the phytonutrient contents of their extracts. A previous study [10] indicated the presence of various



phytochemicals in methanolic Chaya leaves. This suggests that this plant is a good source of flavonoids, saponins, and phenolic compounds. Similarly, research showed the presence of tannins, terpenes, glycosides and alkaloids in *Moringa* leaves [11]. While these studies indicate the potential of locally grown plants to contain phytochemicals that can limit the growth of microbes, there is a need to explore the chemical profile of other plants.

Momordica balsamina Linn is widely distributed across Nigeria and is known for its diverse medicinal and nutritional applications. However, its bioactivities remain largely underexplored [12,13]. *Momordica balsamina* (balsam apple) is an annual vine characterized by tendrils, and it originates from tropical regions in Africa. This plant, belonging to the Cucurbitaceae family, is a significant medicinal and nutritional resource [14]. It exhibits remarkable antimicrobial properties against bacteria, making it a promising source of antimicrobial agents for the treatment of various diseases. On the other hand, *Luffa aegyptica* is a member of the Cucurbitaceae family, which is also referred to as the sponge gourd plant [15]. The fruit of the plant contained a network of fibers that enveloped a substantial quantity of flat, blackish seeds and is mostly found in African countries such as Nigeria and parts of Asia such as India.

Previous studies have indicated the potential of its phytochemical compounds in antibacterial efficacy utilizing different parts of *Luffa aegyptica* and *Momordica balsamina*, such as seeds, fruits, and stem bark, using ethanol and fractions [16,17]. However, little is known about their antibacterial activity using leaf and methanolic extracts as solvents for extraction. The current study aimed to evaluate the phytochemical and antibacterial profile of methanolic leaf extracts of *Momordica balsamina* and *Luffa aegyptica* against clinical bacterial isolates.

2. Materials and Methods

2.1. Sample Collection, Identification and Processing

The leaves of *Momordica balsamina* and *Luffa aegyptica* were collected from the Zaria Local Government Area of Kaduna State and authenticated by a taxonomist in the Herbarium Unit of the Department of Botany Ahmadu Bello University, Zaria. Fresh leaves were washed with distilled water, shade-dried at room temperature, and then ground into a fine powder using a mortar and pestle. The powder samples were stored in clean polythene nylon for further analysis.

2.2. Preparation of Extract

The methanolic extract of the plant leaves was prepared following the protocol outlined by Yusuf et al. [10]. A total of 100 g of plant material was immersed in 500 mL of 100% methanol and subjected to vigorous agitation in conical flasks. The mixture was kept in a well-tighten bottle in dark for 72 h and subsequently filtered through muslin cloth and filter paper. The filtrate was concentrated using a water bath set at 40 °C for 48 h. The resulting crude extract was stored in a sterile container at 4 °C until further use. The extract yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{weight of dry extract}}{\text{weight of dry plant material}} \times 100$$

The extract concentrations were prepared as follows: Briefly, 0.1 g of the methanolic leaf extract of the plant was weighed and added to 10 mL of 10% dimethyl sulfoxide (DMSO). Using a two-fold serial dilution, concentrations of 50 mg/mL, 25 mg/mL, and 12.5 mg/mL were prepared. The various concentrations were labelled and stored in bijou bottles until needed [18].

2.3. Qualitative Phytochemical Screening

The methanolic extract of *M. balsamina* and *L. aegyptica* were tested for the presence of plant secondary metabolites using the following screening procedures described in literature [19], to check for the presence of saponins, flavonoids, tannins, terpenoids, steroids, alkaloids, anthraquinones and cardiac glycosides, as follows:

2.3.1. Test for Saponins

2 mL of the plant extract was taken in a test tube, and exactly 10 mL of distilled water was added. The mixture was vigorously shaken for 30 s and then allowed to stand for 5 min. The presence of saponins was indicated by the formation of a 2 cm layer of foam, which persisted for 10 min.

2.3.2. Test for Flavonoids

The presence of flavonoids in the plant extract was determined by adding 1 mL of NaOH to 3 mL of the extract. The formation of a yellow colouration indicated the presence of flavonoids.

2.3.3. Test for Tannins

The presence of tannins in the plant extract was confirmed by adding 3 drops of 0.1% ferric chloride to 2 mL of the extract. The formation of a brownish-green precipitate indicated the presence of tannins.

2.3.4. Test of Terpenoids

The detection of terpenoids in the plant extract was determined by dissolving 2 mL of chloroform in 5 mL of the extract, followed by the careful addition of 3 mL of concentrated sulfuric acid. The formation of a reddish colouration at the interphase indicated a positive result for terpenoids.

2.3.5. Test for Alkaloids

The presence of alkaloids in the methanolic extract of the plant was determined by adding a few drops of Wagner's reagent (a solution of potassium iodide and iodine) to 2 mL of the extract in a test tube. The formation of an orange-brown precipitate indicated the presence of alkaloids.

2.3.6. Anthraquinone Test

A portion of the plant sample was dissolved in 5 mL of chloroform, shaken, and filtered. An equal amount of 10% ammonia solution was added to the filtrate with continuous shaking. The presence or absence of anthraquinones was indicated by the formation or non-formation of a bright pink colour in the upper aqueous layer.

2.3.7. Cardiac Glycoside Test

A small portion of the plant extract was dissolved in 1 mL of glacial acetic acid (solution containing some traces of ferric chloride), and exactly 1 mL of H₂SO₄ was carefully added to the mixture. The presence or absence of cardiac glycosides was determined by observing the formation or non-formation of a brown ring at the interface.

2.4. Bacterial Isolation and Culturing

Clinical bacterial isolates, specifically *Staphylococcus aureus* (*S. aureus*), *Salmonella typhi* (*S. typhi*), and *Escherichia coli* (*E. coli*) strains were clinical isolates obtained and identified from the Microbiology Laboratory unit at Barau Dikko Teaching Hospital (BDTH) in Kaduna. The samples were collected in nutrient agar slants, appropriately labelled, placed in a cold box, and then transferred to the Microbiology Laboratory at Kaduna State University, Nigeria. Subsequently, the samples were incubated at 37 °C for 24 h for further analysis. An inoculum from an overnight growth culture of the clinical isolates was streaked on freshly prepared plates of Manitol Salt agar, Salmonella-Shigella agar and Eiosin Methylene Blue agar, respectively. All plates were incubated for 24 h at 37 °C to obtain pure cultures of the test isolates.

2.5. Bacterial Identification

A complete loop of the stock cultures of *S. aureus*, *E. coli*, and *S. typhi* were streaked on both blood agar and nutrient agar plates and then incubated for 18–24 h at 37 °C. The morphology of colonies was recorded, and single colonies were selected for basic confirmation tests. These included Gram stain and cultivation onto various differential agars (MacConkey agar, xylose lysine deoxycholate agar, Salmonella-Shigella agar, mannitol salt agar, and eosin methylene blue (EMB) agar). Additionally, biochemical identification tests such as the indole test, oxidase test, coagulase test, catalase test, motility test, Simmon citrate test, MRVP (methyl red, Voges-Proskauer), and TSI (triple sugar iron) were conducted following the procedures documented previously [20]. The identified bacteria were then used in the experiment.

2.6. Antibacterial Susceptibility Test

Antibacterial susceptibility of the plant extract was determined using the agar well diffusion method, following the procedures previously reported [21]. Approximately 0.1 mL of a standardized inoculum from a bacterial suspension was inoculated onto Mueller Hilton agar plates in triplicate measurements. The inoculum was

evenly spread over the surface of the plates using a clean cotton swab stick. After allowing the plates to stand for 10 min, wells with a diameter of 6 mm were created in the agar using a sterile cork borer. A volume of 0.1 mL from each of the various concentrations of the extract (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL) was carefully filled into the respective wells. Additional wells were filled with dimethyl sulfoxide (DMSO) to serve as the negative controls. After allowing the plates to stand for 10 min at room temperature to facilitate the diffusion of extracts into the agar, they were then incubated at 37 °C for 24 h. Following incubation, the zone of inhibition for the growth of each tested bacteria was observed, and the diameter of each zone was measured in millimeters using a ruler. The means and standard deviation were statistically calculated.

2.7. MIC and MBC Tests

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the plant extracts against tested bacteria were carried out following the methodology previously published [22], with some modifications. Briefly, Mueller-Hinton broth (1 mL) was dispensed into five test tubes and autoclaved for sterilization. Afterward, 1 mL of the plant extract was added to the first test tube to create a 50% concentration. A series of twofold dilutions was carried out by transferring 1 mL from one tube to the next, producing concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%. Then, 100 µL of the standardized bacterial culture was introduced into each test tube. The lowest concentration that showed no turbidity, indicating no bacterial growth, was identified as the MIC and recorded. For the MBC test, 50 µL was taken from each MIC tube and placed onto nutrient agar plates, which were incubated overnight at 30 °C to 35 °C. The lowest MIC concentration that showed no visible bacterial growth was considered the MBC.

3. Results and Discussion

The phytochemical Constituents of Methanolic Leaf Extract of *M. balsamina* and *L. aegyptiaca* are shown in (Table 1). Both tested plants showed the presence of alkaloids, flavonoids, saponins, tannins, steroids, terpenes, cardiac glycosides upon qualitative screening, but no anthraquinones detected in both plants. The results of phytochemicals of *M. balsamina* leaf extract align with the result reported in the previous investigation on apple cannell methanolic leaf extract [23]. Also, Adeyeni et al. [16] have documented the presence of saponin, alkaloids, and tannins upon the phytochemical of *L. aegyptiaca* leaf extract collected in Ilorin, Nigeria. Alkaloids manifest their antibacterial properties by interfering with the peptidoglycan constituents within bacterial cells, impeding the synthesis of the cell wall layer and resulting in cellular demise. Also, they serve as DNA intercalators, impeding the activity of bacterial cell topoisomerase enzymes [24]. In addition to these phytonutrients, other studies on *L. aegyptiaca* leaf extract detected the presence of anthraquinones which the present study was not able to detect. Maamoun et al. [11], detected the presence of anthraquinones on ethanolic leaf extract of *L. aegyptiaca* sample analyzed. The disparity between our study with these studies may likely attributed to the types of solvents used during Soxhlet extraction. Indicating that type of solvent yields different results upon preliminary phytochemical screening. The absence of anthraquinones in the plants under study may be notable or expected depending on their phytochemical pathways and evolutionary traits. Anthraquinones are typically synthesized via the polyketide pathway and are common in certain plant families like Polygonaceae and Rubiaceae [25]. Also, their absence could be expected as they produce other protective compounds like flavonoids and alkaloids instead. This absence may also reflect species-specific chemistry or ecological adaptations where the plants prioritize different metabolites for defense or survival.

Table 1. Phytochemical Constituents of Methanolic Leaf Extract of *M. balsamina* and *L. aegyptiaca*.

Bioactive Compounds	<i>M. balsamina</i>	<i>L. aegyptiaca</i>
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Steroids	+	+
Terpenes	+	+
Cardiac glycosides	+	+
Anthraquinones	-	-

Key: +: indicates presence; -: indicates absent.

In line with our results, Valizadeh et al. [26], and Rahmi and Sari [27] reported the presence of saponin during phytochemical screening of *Momordica charantia* leaf extract. Even though different solvents were used between the present study and these previous studies saponin was detected. This suggests that the types of solvents used during extraction do not influence saponin detection. Saponins have been acknowledged for their anti-inflammatory properties, hemolytic activity, and ability to bind cholesterol [15]. In line with the efficacy of saponin on microbial growth, flavonoids also possessed an antibacterial effect against strains of microbes, particularly bacteria. Flavonoids retards bacterial growth by mitigating cellular oxidative stress in bacterial cells making them favourable agents for retarding bacterial growth [15]. The present study has detected the presence of flavonoids in both *M. balsamina* and *L. aegyptiaca* leaf extract which align with what was reported by Yusuf et al. [10] who also reported the presence of flavonoids upon qualitative phytochemical screening. In general, both plants under study belong to the same family (Cucurbitaceae), which explains the presence of similar phytochemical classes. Therefore, we recommend conducting a GC-MS analysis to identify their bioactive compounds in future research.

The antibacterial activity of *M. balsamina* leaf extract shown in Table 2, indicated the extract demonstrated high antibacterial efficacy against *S. typhi* compared with other bacterial isolates at all levels of extract concentrations. *E. coli* has the second inhibition zone after *S. typhi* indicating that the antibacterial activity of the extract was significant ($p < 0.05$) in gram-negative compared to gram-negative bacterial isolates. As such *M. balsamina* leaf extract has more antibacterial efficacy with gram-negative bacteria. However, we observed that the inhibitory activity of the *M. balsamina* extract is concentration-dependent with a varying concentration of 12.5, 25, 50, and 100 mg/mL of the extract. Additionally, the result shows that the extract possessed antibacterial activity against *S. aureus* at a considerably 50 mg/mL concentration. The observed reduced inhibition zone in the *S. aureus* strain may be attributed to its gram-positive nature, characterized by the presence of an extracellular envelope that confers resistance to specific chemicals, including antibiotics. Hence, the variability in susceptibility may stem from distinctions in the permeability barriers of the outer membrane, characterized by lipopolysaccharides in gram-negative bacteria. Furthermore, *E. coli* exhibited greater adaptability in comparison to *S. typhi*, attributed to its inherent resistance to a broad spectrum of antibiotics and its capacity to form biofilms on surfaces. This biofilm formation renders the cells less susceptible to medicinal antimicrobial agents at specific concentrations [28,29]. The variation in antibacterial activity of plant extracts against Gram-positive and Gram-negative bacteria is largely influenced by the structural defenses of the bacteria, the chemical properties of the bioactive compounds, and the specific interactions between these compounds and bacterial cells. Understanding these factors helps explain why some plant extracts are more effective against one group of bacteria over the other.

Table 2. Antibacterial activity of Methanolic Leaf Extract of *M. balsamina* against Bacterial Isolates.

Bacteria	Zones of Inhibition (mm) at Different Concentration (mg/mL)				
	100	50	25	12.5	Ciprofloxacin
<i>Staphylococcus aureus</i>	12.23 ± 0.31 ^c	10.07 ± 0.15 ^c	4.73 ± 0.25 ^b	2.70 ± 0.44 ^b	35.43 ± 0.31 ^c
<i>Salmonella typhi</i>	20.90 ± 0.10 ^a	17.07 ± 0.25 ^a	10.73 ± 0.50 ^a	6.77 ± 0.21 ^a	39.13 ± 0.47 ^b
<i>Escherichia coli</i>	17.67 ± 0.15 ^b	15.27 ± 0.25 ^b	10.03 ± 0.32 ^a	6.10 ± 0.36 ^a	42.47 ± 0.15 ^a

Values are mean ± standard deviation, means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at ($p < 0.05$).

Regarding *L. aegyptiaca*, the results show that it has more antibacterial against all the clinical bacterial isolates tested (Table 3). Even at a lower concentration of 12.5 mg/mL *L. aegyptiaca* extract has good antibacterial activity against *S. typhi* strain compared with other strains of organism. However, at 25 mg/mL and 12.5 mg/mL concentrations of *L. aegyptiaca* extract the zone of inhibition between *S. aureus* and *E. coli* strains is not statistically significant ($p < 0.05$). Indicating that twice the concentration of the extract is needed to exert the antibacterial efficacy of *S. aureus* by the plant extract. In the control group *E. coli* has the highest zone of inhibition compared with other strains. This indicated that the organism has more sensitivity to ciprofloxacin than *S. aureus* and *S. typhi*. A similar study on antibacterial activity has also recorded similar trends in antibacterial activity against *E. coli* [30]. The level of inhibition in *S. typhi* is probably due to the presence of alkaloids and flavonoids which have been previously summarized to possess antimicrobial activity through inhibition of cellular membrane synthesis and as well acting as bacterial cytotoxins [31].

Table 3. Antibacterial activity of Methanolic Leaf Extract of *L. aegyptiaca* against Bacterial Isolates.

Bacteria	Zones of Inhibition (mm) at Different Concentration (mg/mL)				
	100	50	25	12.5	Ciprofloxacin
<i>S. aureus</i>	6.77 ± 0.25 ^c	5.17 ± 0.12 ^c	3.90 ± 0.10 ^b	2.50 ± 0.00 ^b	35.43 ± 0.31 ^c
<i>S. typhi</i>	11.63 ± 0.35 ^a	9.80 ± 0.30 ^a	6.73 ± 0.25 ^a	3.67 ± 0.40 ^a	39.13 ± 0.47 ^b
<i>E. coli</i>	9.87 ± 0.64 ^b	6.17 ± 0.15 ^b	4.13 ± 0.23 ^b	2.70 ± 0.26 ^b	42.47 ± 0.15 ^a

Values are mean ± standard deviation, means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at ($p < 0.05$).

The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the methanol leaf extracts of *M. balsamina* and *L. aegyptiaca* against bacterial isolates are presented in Table 4. The lowest MIC was 12.5 mg/mL and the highest was 50 mg/mL, while lowest MBC was 25 mg/mL and highest was 50 mg/mL respectively. In both plant extracts *S. typhi* has the highest response with strong antibacterial activity at a lower concentration of 12.5 mg/mL extract. *E. coli* and *S. aureus* responded to the antibacterial of the two plant extracts at 25 mg/mL for *M. balsamina*. Previous studies have indicated the potentiality of *M. balsamina* to inhibit the growth of microbes even at a considerably low concentration of 25 mg/mL [32,33]. The antibacterial efficacy recorded in both plant extracts may likely be due to the presence of bioactive phytochemical compounds such as cardiac glycosides and tannins which disrupt bacterial growth. Cardiac glycosides exert antibacterial activity by inhibiting bacterial ATPase, disrupting cellular ion gradients critical for bacterial viability [34]. Tannins, on the other hand, display antibacterial effects by precipitating proteins in bacterial cell walls, leading to structural alterations and membrane destabilization [7]. Generally, Gram-positive bacteria have a thick, exposed peptidoglycan layer that is easily targeted by antibiotics, making them more susceptible. In contrast, Gram-negative bacteria possess an additional outer membrane containing lipopolysaccharides and porins, which limit antibiotic entry. This outer membrane, along with efficient efflux pumps and periplasmic beta-lactamases, provides Gram-negative bacteria with greater resistance. Their complex cell wall structure and defense mechanisms make Gram-negative bacteria generally more resistant to many antibiotics [35].

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Plant Extracts.

Bacteria	MIC (mg/mL)		MBC (mg/mL)	
	<i>M. balsamina</i>	<i>L. aegyptiaca</i>	<i>M. balsamina</i>	<i>L. aegyptiaca</i>
<i>S. aureus</i>	25	50	50	50
<i>S. typhi</i>	12.5	12.5	25	50
<i>E. coli</i>	25	25	25	50

The MBC of the test organism indicated bacterial growth retards at approximately 25 to 50 mg/mL concentrations of *M. balsamina* extract indicating the plant has more inhibitory compared with *L. aegyptiaca*. *S. typhi* has the lowest MBC (Table 4) compared with other bacterial isolates against *M. balsamina* leaf extract. The lower MBC observed in *S. typhi* is presumably attributable to the characteristics inherent in its bacterial cell membrane. Also, the variations in antibacterial efficacy between *Luffa aegyptiaca* and *Momordica balsamina* are attributed to differences in their chemical compositions and the concentrations of individual compounds. While our qualitative analysis has identified the major phytochemical classes, further in-depth investigation using GC-MS is essential to elucidate the specific compounds, as each phytochemical class comprises thousands of bioactive constituents.

Concurrently, in both bacterial isolates, we have found that *L. aegyptiaca* exerts its antibacterial efficacy at a considerably at 25 to 50 mg/mL concentration. This indicates that *M. balsamina* has more antibacterial effects compared with *L. aegyptiaca*. The efficacy of medicinal plant in exerting its antibacterial property depends on the concentration of the extract. Scientific investigations shown that these natural phytochemical substances exert both direct and indirect impacts on human, animal, and microbial physiology. Some phytochemical substances possess the capability to inhibit or eliminate harmful microorganisms via distinct mechanisms of action [36]. Finally, the results of our current study reveal the main active ingredients in the leaves of these two plants. The study recommends conducting further research to identify these active compounds and determine their mechanisms of action on bacteria using advanced techniques and approaches, such as Metabolomics and Proteomics, Transcriptomics (RNA-Sequencing), Molecular Docking and in Silico Modeling, Fluorescence Microscopy and Confocal Laser Scanning Microscopy, Atomic Force Microscopy (AFM), Flow Cytometry, and Mass Spectrometry-Based Metabolic Profiling. These methods will help us understand how plant-derived compounds

combat bacteria, guiding the development of new, effective antimicrobial agents from these plants. Metabolomics can provide a detailed profile of the metabolites present in the plant extracts, helping to pinpoint which specific compounds contribute to their antibacterial effects. Molecular docking, on the other hand, allows researchers to simulate the interaction between these bioactive compounds and bacterial targets, giving insights into their mode of action. By combining these approaches, the study would gain a deeper understanding of how the extracts work at a molecular level, enhancing the scientific validity of the findings.

4. Conclusion

This research successfully identified a broad spectrum of phytochemicals, including alkaloids, flavonoids, saponins, steroids, tannins, cardiac glycosides, terpenoids, and carbohydrates in the methanolic extracts of *Momordica balsamina* and *Luffa aegyptiaca* leaves, although anthraquinones were notably absent. The study highlighted the significant antibacterial potential of these extracts, particularly against the Gram-negative bacterium *Salmonella typhi*, demonstrating substantial inhibition zones even at a low concentration of 25 mg/mL. The extracts exhibited high phytonutrient content and showed remarkable efficacy against both Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*S. typhi* and *Escherichia coli*), with a pronounced effectiveness against Gram-negative strains. These findings suggest that the methanolic extracts of *M. balsamina* and *L. aegyptiaca* could be promising candidates for the development of novel antibacterial agents to combat infectious diseases, particularly those caused by Gram-negative pathogens. Future studies are recommended to focus on the isolation and characterization of individual bioactive compounds within these extracts.

Author Contributions: M.S.G., B.I., and F.M.: methodology; M.S.G.: experiments; M.S.G., B.I., and F.M.: conceptualization and writing—original draft; B.I., and F.M.: visualization, E.M.A.: writing—review, editing, evaluation, correspondence, revision, and consultations. The draft manuscript was read and approved by all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This particular research did not receive any funding from any government or non-government organizations.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available upon request from the 1st authors (M.G., email: kasumsbio180016@kasu.edu.ng).

Acknowledgments: The authors would like to thank the Department of Biological Sciences, Faculty of Life Sciences, College of Science, Computing, and Engineering, Kaduna State University, 800283, Kaduna State, Nigeria, for providing research and laboratory facilities.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sulieman, A.M.E.; Abdallah, E.M.; Alanazi, N.A.; et al. Bioactive profiling of *Rumex vesicarius* L. from the Hail region of Saudi Arabia: A study on its phytochemical and elemental analysis, antibiofilm, antibacterial, antioxidant properties, and molecular docking analysis. *Front. Microbiol.* **2024**, *15*, 1421143.
2. Abdallah, E.M. Plants: An alternative source for antimicrobials. *J. Appl. Pharm. Sci.* **2011**, *1*, 16–20.
3. Brüßow, H. The antibiotic resistance crisis and the development of new antibiotics. *Microb. Biotechnol.* **2024**, *17*, e14510.
4. Mendelson, M.; Lewnard, J.A.; Sharland, M.; et al. Ensuring progress on sustainable access to effective antibiotics at the 2024 UN General Assembly: A target-based approach. *Lancet* **2024**, *403*, 2551–2564.
5. Aumeeruddy, M.Z.; Mahomoodally, M.F. Traditional herbal therapies for hypertension: A systematic review of global ethnobotanical field studies. *S. Afr. J. Bot.* **2020**, *135*, 451–464.
6. Yousofvand, N.; Moloodi, B. An overview of the effect of medicinal herbs on pain. *Phytother. Res.* **2023**, *37*, 1057–1081.
7. Parham, S.; Kharazi, A.Z.; Bakhsheshi-Rad, H.R.; et al. Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants* **2020**, *9*, 1309.
8. Bashir, M.; Ibrahim, A.; Bilyaminu, M.; et al. Phytochemical screening and antibacterial activity of leaf and stem bark extracts of *Adansonia digitata* on *E. coli*, *S. aureus* and *S. typhi*. *Microbes Infect. Dis.* **2022**, *3*, 217–223.
9. Li, Z.; Xia, A.; Li, S.; et al. The pharmacological properties and therapeutic use of bitter melon (*Momordica charantia* L.). *Curr. Pharmacol. Rep.* **2020**, *6*, 103–109.
10. Yusuf, A.; Abubakar, J.; Lawal, A. Phytochemicals Screening and Nutritional Profile of *Cnidoscopus aconitifolius* Leaves collected in Birnin Kebbi, Nigeria. *Sch. Int. J. Biochem.* **2022**, *5*, 85–89.
11. Maamoun, A.A.; El-Akkad, R.H.; Farag, M.A. Mapping metabolome changes in *Luffa aegyptiaca* Mill fruits at different maturation stages via MS-based metabolomics and chemometrics. *J. Adv. Res.* **2021**, *29*, 179–189.

12. Okusanya, O.; Ola-Adams, B.; Bamidele, J. Variations in size, leaf morphology, and fruit characters among 25 populations of *Luffa aegyptiaca*. *Can. J. Bot.* **1981**, *59*, 2618–2627.
13. Kadiri, O.J.; Okafor, S.I.; Ogaji, J.I. Safety and health benefits profile studies of leaf extracts of *Momordica balsamina* Linn (Cucurbitaceae) found in North Central Nigeria. *GSC Biol. Pharm. Sci.* **2020**, *11*, 278–286.
14. Coleman, M.I.; Khan, M.; Gbodossou, E.; et al. Identification of a novel anti-HIV-1 protein from *Momordica balsamina* leaf extract. *Int. J. Environ. Res. Public Health* **2022**, *19*, 15227.
15. Harfiani, E.; Pradana, D.C.; Yusmaini, H. A Review on the Phytochemical and Pharmacological Activities of *Luffa acutangula* (L.) Roxb. *Pharm. J. Farm. Indones.* **2020**, *17*, 396–406.
16. Adeyeni, E.; Ayodele, E.; Adedosu, T. Phytochemical Constituents and Antimicrobial Activities of Ethanolic Extract of *Luffa Cylindrica* Seed. *Ilorin J. Sci.* **2020**, *7*, 127–143.
17. Mashiane, P.; Manhivi, V.E.; Shoko, T.; et al. Cooking African pumpkin leaves (*Momordica balsamina* L.) by stir-frying improved bioactivity and bioaccessibility of metabolites—Metabolomic and chemometric approaches. *Foods* **2021**, *10*, 2890.
18. Hassan Cheong, N.D.; Zakaria, L.A.; Yusof, H. Qualitative Phytochemical Screening and Antibacterial Properties of *Momordica charantia* Methanolic Extract Against Selected Bacterial Strains. *Malays. J. Med. Health Sci.* **2022**, *18*, 154–161.
19. Mishra, B.B.; Tiwari, V.K. Natural products: An evolving role in future drug discovery. *Eur. J. Med. Chem.* **2011**, *46*, 4769–4807.
20. Muribeca, A.d.J.; Gomes, P.W.P.; Paes, S.S.; et al. Antibacterial Activity from *Momordica charantia* L. Leaves and Flavones Enriched Phase. *Pharmaceutics* **2022**, *14*, 1796.
21. Doğaroğlu, Z.G.; Uysal, Y.; Çayla, Z.; et al. Antibacterial and phytotoxicological properties assessment of *Momordica charantia* extract-based ZnO nanoparticles. *J. Sci. Food Agric.* **2024**, *104*, 2851–2861.
22. Abdallah, E.M. Antibacterial activity of *Hibiscus sabdariffa* L. calyces against hospital isolates of multidrug resistant *Acinetobacter baumannii*. *J. Acute Dis.* **2016**, *5*, 512–516.
23. Abdulhamid, A.; Jega, S.; Sani, I.; Bagudo, A.; Abubakar, R. Phytochemical and antibacterial activity of *Momordica balsamina* leaves crude extract and fractions. *Drug Discov.* **2023**, *17*, e11dd1012.
24. Egamberdieva, D.; Jabborova, D.; Babich, S.; et al. Antimicrobial activities of herbal plants from Uzbekistan against human pathogenic microbes. *Environ. Sustain.* **2021**, *4*, 87–94.
25. Nallakaruppan, N.; Thiagarajan, K. In vitro elicitation of anthraquinones—A review. *Plant Cell Tissue Organ Cult.* **2024**, *156*, 70.
26. Valizadeh, M.; Beigomi, M.; Fazeli-Nasab, B. Antibacterial and Anti biofilm effects of ethanol and acetone leaf extract of *Momordica charantia* and *Tecomella undulata* against *Acinetobacter baumannii*. *Int. J. Adv. Biol. Biomed. Res.* **2020**, *8*, 403–418.
27. Rahmi, M.; Sari, T. Antibacterial activity of ethanol extract, n-hexane, ethyl acetate and butanol fraction of *Momordica charantia* L. seed against *Staphylococcus epidermidis*. *J. Phys. Conf. Ser.* **2021**, *1918*, 052013.
28. Sherekar, P. Antimicrobial agent from Plants and Herbs: A systemic Review. *Res. J. Pharmacogn. Phytochem.* **2021**, *13*, 179–181.
29. Samadov, B.S. The use of the medicinal plant *Momordica charantia* L in folk medicine. *Asian J. Pharm. Biol. Res.* **2022**, *11*.
30. Dalkılıç, S.; Dalkılıç, L.K.; Küçüktüfekçi, M.C.; et al. Determination of cytotoxic, apoptotic, necrotic, antimicrobial and antioxidant activities of *Aloe vera* and *Abies cilicica* subsp. *cilicica*. *Int. J. Plant Based Pharm.* **2023**, *3*, 165–175.
31. Oyelere, S.F.; Ajayi, O.H.; Ayoade, T.E.; et al. A detailed review on the phytochemical profiles and anti-diabetic mechanisms of *Momordica charantia*. *Heliyon* **2022**, *8*, e09253.
32. Choudhary, B.; Berwal, M.; Ram, H.; et al. *Momordica balsamina* L.: An unexploited vegetable crop rich in medicinal and nutritional properties. *J. Agric. Ecol.* **2022**, *14*, 84–92.
33. Dien, T.N.; Thi, H.N. Antibacterial activity of silver nanoparticle LED-synthesized using *Citrus maxima* peels. *Int. J. Plant Based Pharm.* **2023**, *3*, 141–147.
34. Shahrajabian, M.H.; Cheng, Q.; Sun, W. The most important medicinal herbs and plants in traditional Chinese and Iranian medicinal sciences with antioxidant activities. *Lett. Drug Des. Discov.* **2023**, *20*, 1171–1184.
35. Alegun, O.; Pandeya, A.; Cui, J.; et al. Donnan potential across the outer membrane of gram-negative bacteria and its effect on the permeability of antibiotics. *Antibiotics* **2021**, *10*, 701.
36. Sadeek, A.; Abdallah, E.M. Phytochemical compounds as antibacterial agents a mini review. *Saudi Arab. Glob. J. Pharmaceut. Sci.* **2019**, *53*, 555720.