



Plant-Derived Phytochemicals from *Moringa oleifera* and *Chenopodium album* as Inhibitors of *Staphylococcus aureus*

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Received: 3 September 2026; Revised: 10 October 2026; Accepted: 13 October 2026; Published: 23 October 2026

Abstract: *Staphylococcus aureus* is an important bacterial pathogen associated with human, animal, and environmental infections, creating a need to investigate alternative sources of antibacterial compounds. This study evaluated the antibacterial activity of *Moringa oleifera* and *Chenopodium album* leaf extracts against *S. aureus* isolated from poultry-shed soil in Sylhet, Bangladesh. Three bacterial isolates were characterized using microbiological and biochemical methods and further evaluated by PCR amplification of the species-specific *nuc* gene. Aqueous, ethanolic, and methanolic leaf extracts were prepared and screened using the disc diffusion method. Qualitative phytochemical screening was also performed. Among the tested extracts, the methanolic extract of *M. oleifera* produced the largest inhibition zone (34.0 ± 0.5 mm), followed by the methanolic extract of *C. album* (24.0 ± 0.6 mm). The ethanolic extracts produced inhibition zones of 20.0 ± 1.0 mm and 22.0 ± 0.4 mm for *M. oleifera* and *C. album*, respectively, whereas no measurable inhibition was observed with the aqueous extracts. The vancomycin reference control produced a 22 mm inhibition zone under the experimental conditions. Qualitative phytochemical screening indicated the presence of several classes of secondary metabolites, including alkaloids, tannins, saponins, flavonoids, coumarins, quinones, phenols, cardiac glycosides, steroids, and triterpenoids. These findings provide preliminary *in vitro* evidence of antibacterial activity in selected plant extracts and support further investigation using quantitative phytochemical analysis, MIC and MBC determination, identification of active compounds, and broader bacterial isolate panels.

Keywords: antibacterial; *Staphylococcus aureus*; Phytochemical; *Moringa oleifera*; *Chenopodium album*

1. Introduction

Staphylococcus aureus is a commensal, coagulase-positive bacterium that colonizes a wide range of hosts, including humans, animals, and environmental reservoirs. It is responsible for a broad spectrum of infections, ranging from mild skin conditions to severe systemic diseases such as sepsis, pneumonia, osteomyelitis, and endocarditis. In agricultural settings, *S. aureus* is associated with septic arthritis in poultry and mastitis in dairy cattle [1], leading to significant economic losses. The pathogenicity of *S. aureus* is attributed to multiple virulence factors, including toxin production and increasing resistance to several classes of antibiotics [2].

The emergence of antibiotic-resistant strains has intensified the search for alternative antimicrobial agents [3]. Plant-derived compounds have gained considerable attention as potential sources of natural antimicrobial agents, particularly in traditional medicine and natural product-based drug discovery [4,5]. Medicinal plants produce a wide range of bioactive secondary metabolites with antimicrobial properties and have historically been used for the treatment of various infectious diseases [6]. In this context, the present study evaluated the antibacterial activity of two plant species, *Moringa oleifera* and *Chenopodium album*, using ethanol, methanol, and aqueous extracts against *S. aureus*.

Moringa oleifera is one of the most widely cultivated species of the genus *Moringa* and belongs to the family Moringaceae. The plant is recognized for its considerable nutritional and medicinal value and is widely utilized in traditional and modern healthcare systems [7]. Commonly known as the drumstick tree, horseradish tree, or benoil tree, *M. oleifera* is extensively distributed throughout tropical and subtropical regions of the world [8]. Various parts of the plant, including its leaves, pods, and seeds, are rich sources of proteins, vitamins, minerals, and



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bioactive compounds that contribute to its nutritional significance. In particular, the leaves contain substantial amounts of calcium, phosphorus, iron, vitamins A and C, and other essential nutrients [9]. Owing to its rich phytochemical profile and nutritional composition, *M. oleifera* has attracted considerable scientific interest for its potential health-promoting properties.

Chenopodium album, commonly known as bathua, fat-hen, vastukah, or chakvit, belongs to the genus *Chenopodium* and the family *Chenopodiaceae* [10]. Although frequently regarded as a weed in agricultural fields, the plant is widely consumed as a leafy vegetable in many regions and serves as a valuable source of nutrients. The tender shoots and leaves can be consumed raw in salads or cooked as vegetables and are incorporated into various traditional food preparations. Previous studies have reported that *C. album* contains diverse bioactive constituents, including flavonoids, alkaloids, phenolic compounds, saponins, and other secondary metabolites that may contribute to its biological activities [11]. Traditionally, the plant has been used for the management of several ailments, and its leaves have been employed in folk medicine for wound care, burn treatment, and relief of various inflammatory conditions.

Although the antibacterial properties of *Moringa oleifera* and *Chenopodium album* have been investigated individually, comparative studies evaluating these two plants under a common experimental framework remain limited. In particular, limited information is available on the comparative antibacterial activity of aqueous, ethanolic, and methanolic leaf extracts of both species against molecularly confirmed *Staphylococcus aureus* recovered from poultry-shed environments. Therefore, the novelty of the present study lies in the side-by-side evaluation of two locally accessible medicinal plants using three extraction solvents, combined with phenotypic characterization and molecular confirmation of the test organism through amplification of the species-specific *nuc* gene. The study further compares the phytochemical profiles and antibacterial activities of the different extracts under identical experimental conditions, allowing the effects of plant species and extraction solvent on antibacterial activity to be examined within the same experimental framework. This provides locally relevant evidence for further investigation of these plant materials as sources of antibacterial compounds in veterinary and agricultural contexts.

Accordingly, the objectives of this study were to (i) isolate and molecularly confirm *S. aureus* from poultry-shed soil; (ii) compare the antibacterial activity of aqueous, ethanolic, and methanolic leaf extracts of *Moringa oleifera* and *Chenopodium album* under identical disc-diffusion conditions; and (iii) qualitatively screen the extracts for major phytochemical classes and interpret their occurrence in relation to the observed antibacterial activity without assigning the activity to specific compounds.

2. Materials and Methods

2.1. Isolation of *Staphylococcus aureus*

Soil samples were collected aseptically from poultry-shed areas of the Government Poultry Breeding and Development Farm, Sylhet, Bangladesh, and transported to the laboratory in sterile plastic bags for microbiological analysis. For bacterial isolation, 1 g of each soil sample was suspended in 9 mL of sterile distilled water, and serial ten-fold dilutions were prepared up to 10^{-3} . Mannitol Salt Agar (MSA) was used as the selective medium and sterilised by autoclaving at 121 °C and 15 psi for 15–20 min. Aliquots of the diluted samples were streaked onto MSA plates and incubated at 37 °C for 24 h. Colonies showing characteristic growth and yellow colouration on MSA due to mannitol fermentation were selected as presumptive *Staphylococcus* isolates for further characterisation.

2.2. Microscopic Characterization by Gram's Staining Method

Gram staining was performed according to the method described by [12] to determine the Gram reaction, morphology, and arrangement of the bacterial isolates. The isolates appeared as Gram-positive cocci arranged in grape-like clusters and stained purple, characteristics consistent with *Staphylococcus aureus*.

2.3. Biochemical Tests

After isolation, the bacterial isolates were subjected to biochemical tests for presumptive identification. The principles and procedures of the tests are described below.

2.3.1. Catalase Test

Using sterile technique, a small amount of the organism was transferred from a well-isolated 18–24 h old colony onto a clean glass slide. One drop of 3% H_2O_2 was then added to the bacterial material without mixing.

The reaction was observed immediately for bubble formation. Rapid effervescence indicated a positive catalase reaction, whereas the absence of bubble formation indicated a negative reaction [13].

2.3.2. Citrate Utilization Test

This test determines whether an organism can use citrate as a carbon source. Bromothymol blue is used as a pH indicator. When the pH increases above 7.6, the indicator changes from green to blue, indicating citrate utilization.

2.3.3. MR Test

Five milliliters of sterile MR-VP broth were mixed with a small amount of pure test organism, and the mixture was then grown for twenty-four hours at 37 degrees Celsius. Following incubation, five drops of methyl red solution were added, mixed, and the color output was then observed. Red color development denoted a favorable outcome, whereas yellow color development denoted a poor outcome [14].

2.3.4. Voges-Proskauer (V-P) Test

Pure cultures were inoculated into 5 mL of sterile MR-VP broth and incubated overnight at 37 °C. Following incubation, 6 mL of 5% α -naphthol and 0.2 mL of 40% potassium hydroxide containing 0.3% creatine were added per mL of the broth culture. The culture was shaken thoroughly for 3–4 min and then allowed to stand for 15 min. Development of a pink-red color was interpreted as a positive reaction, whereas the absence of color was interpreted as a negative reaction [13].

2.3.5. Oxidase Test

One or two drops of 1% Kovács oxidase reagent were applied to the bacterial culture. The reagent was applied without flooding or inverting the plate. A dark-purple color appearing within 5–10 s was interpreted as a positive oxidase reaction [13].

2.3.6. Indole Test

Following incubation of the culture for at least 24 h, Kovács reagent was added to the culture medium. Formation of a red or pink layer at the surface was interpreted as a positive indole reaction, whereas the absence of a red layer was interpreted as a negative reaction.

2.3.7. Urease Test

The isolates were inoculated into urea broth and incubated for 24 h at 37 °C. A reddish-pink or red color was interpreted as a positive urease reaction, whereas the absence of a color change was interpreted as a negative reaction.

2.4. Molecular Identification of *Staphylococcus aureus* by PCR

2.4.1. DNA Extraction

Genomic DNA was extracted from presumptive *Staphylococcus aureus* isolates using the boiling method. Fresh bacterial colonies were suspended in sterile nuclease-free water and heated at 95 °C for 10 min. The suspension was then chilled on ice and centrifuged at 10,000 rpm for 5 min. The supernatant containing bacterial DNA was collected and stored at –20 °C until further use.

2.4.2. PCR Amplification of *nuc* Gene

Molecular confirmation of *Staphylococcus aureus* was performed by amplification of the species-specific *nuc* gene using polymerase chain reaction (PCR). The primer sequences used for amplification are listed in Table 1.

Table 1. Primers used for amplification of the *Staphylococcus aureus*-specific *nuc* gene.

Target Gene	Primer Sequence (5'-3')	Amplicon Size (bp)	Reference
<i>nuc</i>	F: GCG ATT GAT GGT GAT ACG GTT	278 bp	[15]
	R: AGC CAA GCC TTG ACG AAC TAA AGC		

PCR amplification was carried out in a 25 μ L reaction mixture containing PCR master mix, forward and reverse primers, template DNA, and nuclease-free water. The amplification conditions consisted of initial

denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 5 min.

2.4.3. Agarose Gel Electrophoresis

The amplified PCR products were separated on a 1.5% agarose gel prepared in 1× TBE buffer and stained with ethidium bromide/safe DNA stain. A 100 bp DNA ladder was used as the molecular size marker. Electrophoresis was performed at 100 V for approximately 45 min, and the amplified products were visualised under a UV transilluminator. The presence of an approximately 278-bp amplicon, consistent with the expected product size of the *nuc*-specific primer pair, was considered presumptive evidence of *Staphylococcus aureus*.

2.5. Phytochemical Screening of Plant Sample

2.5.1. Collection and Preparation of Plant Extracts

Mature leaves of *Moringa oleifera* and *Chenopodium album* were collected from the campus premises of Sylhet Agricultural University, Sylhet, Bangladesh, at approximately 24°54'33" N, 91°54'07" E (24.909221° N, 91.901986° E), during February 2024. The plant specimens were taxonomically authenticated by the Department of Plant and Environmental Biotechnology, Sylhet Agricultural University, where official specimen voucher numbers were deposited (Voucher Nos.: SAU-PEB-2024/MO-012 for *M. oleifera* and SAU-PEB-2024/CA-015 for *C. album*). Fresh, healthy leaves were separated, thoroughly washed, surface-sterilised, and rinsed with distilled water. The leaves were oven-dried at 45 °C for 72 h until a constant weight was obtained and subsequently ground into a fine powder using a laboratory blender.

Twenty grams of powdered leaf material from each plant were soaked separately in 100 mL of ethanol, methanol, and distilled water in sterile conical flasks and kept for 72 h. The extracts were filtered through filter paper to obtain clear filtrates, and the extraction process was repeated two to three times. The combined filtrates were concentrated by evaporation in a hot-air oven at 45 °C. The dried extracts were then stored under refrigeration until further use [4].

The extraction yield was calculated as the weight of dried extract obtained relative to the initial dry weight of plant material using the equation: extraction yield (%) = [weight of dried extract (g)/initial dry weight of plant material (g)] × 100. Twenty grams of powdered leaf material from each plant were used for each extraction. The extraction yields obtained for *Moringa oleifera* ranged from 12–18% for the aqueous extract, 8–15% for the ethanolic extract, and 10–18% for the methanolic extract. For *Chenopodium album*, the corresponding extraction yields ranged from 10–16%, 10–16%, and 7–17% for the aqueous, ethanolic, and methanolic extracts, respectively.

2.5.2. Phytochemical Analysis of Plant Extracts

The aqueous, methanolic, and ethanolic extracts of *Moringa oleifera* (Moringa) and *Chenopodium album* (Bathua) were qualitatively screened for the presence of phytochemicals, alkaloids, tannins, saponins, flavonoids, coumarins, quinones, phenols, cardiac glycosides and steroids [16].

Alkaloids Test

Alkaloids were detected using Wagner's test, in which a few drops of Wagner's reagent were added to 2 mL of the sample in a test tube. The formation of a reddish-brown precipitate was taken as a positive indication of alkaloid presence [16].

Tannins Test

Ferric chloride is used to detect the presence of tannins. This involves taking a 2 mL sample and adding a few drops of 5% ferric chloride solution to it in a test tube. Tannins are indicated by a blackish blue, brownish green, or blackish green color [17]. The presence of a blackish-blue color suggested gallic tannins, while the presence of a greenish-blackish color indicated catechol tannins [18].

Saponins Test

To create a stable, long-lasting foam, 2 milliliters of plant extract and 3 milliliters of distilled water were agitated vigorously. Emulsion development is a sign that saponins are present.

Flavonoids Test

The presence or absence of flavonoids was determined by the lead acetate test. One milliliter of the extract was mixed with a few drops of a 10% lead acetate solution. When a yellow-colored precipitate appears, flavonoids are present [19].

Coumarins Test

For the coumarin test, 1 mL of aqueous plant extract was placed in a test tube, followed by 1 mL of 10% NaOH. The formation of a yellow colour was interpreted as a positive reaction for coumarins [20].

Quinones Test

One millilitre of plant extract was placed in a clean test tube, and 1 mL of concentrated HCl was added. The formation of a yellow precipitate was interpreted as a positive reaction for quinones [20].

Steroids and Triterpenoids Test

Presence of steroids and triterpenoids was confirmed through the Salkowski test. 2 milliliters of crude extract and chloroform were combined in a test tube for this test. After adding a few drops of strong H₂SO₄ acid, it is thoroughly mixed and left to stand for a while. A brown ring formed at the junction of 2 layers. Steroids were indicated when the lower layer turned red, while the presence of triterpenoids was shown by the creation of a yellow layer [21].

Phenols Test

One milliliter of plant extract was mixed with a few drops of a 5% ferric chloride solution in a test tube. When phenol is present, the coloration becomes blue, green, reddish-brown, or dark violet. A clear, yellow, or orange solution with no precipitate gives a negative result [19].

Cardiac Glycosides Test

To find out if cardiac glycosides were present, the Keller-Kiliani test was used. One millilitre of concentrated H₂SO₄, one millilitre of glacial acetic acid, and one or two drops of 5% ferric chloride are added to two millilitres of the sample in a test tube. A brown ring formed between the layers, signifying the presence of cardiac steroidal glycosides [22].

2.6. Antibacterial Screening of Plant Extracts against Isolated Bacteria

2.6.1. Antibacterial Screening of Plant Extracts

The antibacterial activities of the aqueous, methanolic, and ethanolic leaf extracts of *Moringa oleifera* and *Chenopodium album* were evaluated against the three molecularly confirmed *Staphylococcus aureus* isolates (PS A, PS B, and PS C) using the Kirby-Bauer disc-diffusion method [23].

2.6.2. Inoculum Preparation

The bacterial isolates used for antibacterial testing were PS A, PS B, and PS C. Each plant extract was tested once against each isolate, resulting in three isolate-level observations per extract treatment (n = 3). These observations represent three distinct bacterial isolates rather than technical replicates of the same isolate.

2.6.3. Inoculation of Test Plates

Approximately 15 mL of Mueller-Hinton agar was poured into sterile Petri dishes and allowed to solidify. The surface of each agar plate was uniformly inoculated with the prepared bacterial culture using a sterile cotton swab [4].

2.6.4. Application of Extract-Impregnated Discs

The dried plant extracts were reconstituted to a final concentration of 10 mg/mL in their respective solvents (methanol, ethanol, or distilled water). Sterile filter-paper discs of 6 mm diameter were impregnated with 20 µL of each extract solution, corresponding to 0.20 mg of crude extract per disc. The discs were aseptically placed on

the inoculated Mueller-Hinton agar plates using sterile forceps. A vancomycin reference control containing 27 µg of vancomycin per disc was used for comparison with the plant extracts. The plates were allowed to stand for 15 min to facilitate diffusion and were subsequently incubated at 37 °C for 18–22 h [4].

2.6.5. Reading of Plates and Interpretation of Results

Following incubation, the plates were examined for clear zones of growth inhibition surrounding the discs. The diameter of each inhibition zone, including the diameter of the disc, was measured in millimetres using a ruler. The measured values were recorded for statistical analysis. The presence of a measurable clear zone surrounding an extract-impregnated disc was considered indicative of antibacterial activity under the experimental conditions.

2.7. Statistical Analysis

For each plant-extract treatment, inhibition-zone diameters were summarised as mean ± standard deviation (SD) based on three isolate-level observations corresponding to PS A, PS B, and PS C (n = 3). Each isolate was tested once against each extract, without additional technical replicate discs. Descriptive statistics were used to summarise the observed inhibition-zone diameters.

3. Results

3.1. Isolation of *Staphylococcus aureus*

Three bacterial isolates, designated PS A, PS B, and PS C, were recovered from the soil sample on Mannitol Salt Agar (MSA). All isolates exhibited colony characteristics consistent with *Staphylococcus aureus*. Colony morphology was recorded after 24 and 48 h of incubation, and the observed characteristics are presented below (Figure 1).

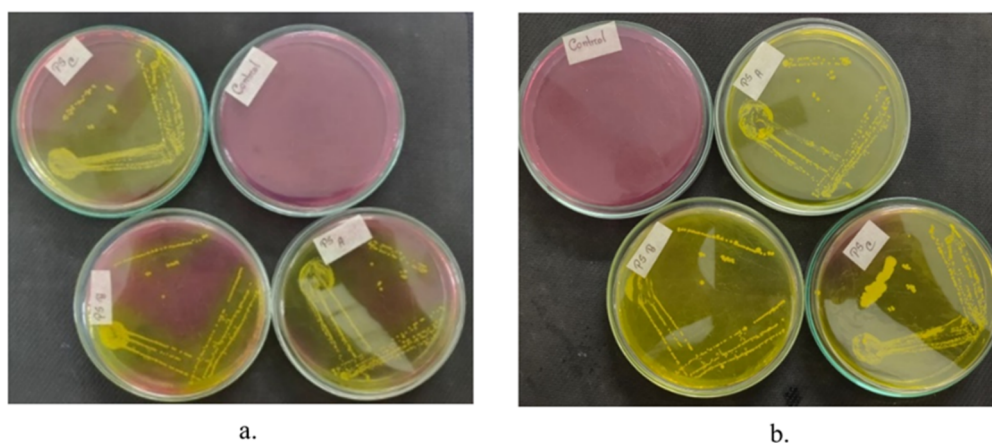


Figure 1. Morphological appearance of bacterial colonies on MS agar medium following incubation for (a) 24 h and (b) 48 h.

3.2. Microscopic Characterization by Gram Staining

Microscopic examination of the Gram-stained isolates revealed purple-stained cocci arranged in grape-like clusters, indicating that the isolates were Gram-positive. These morphological characteristics were consistent with those of *Staphylococcus aureus* (Figure 2).

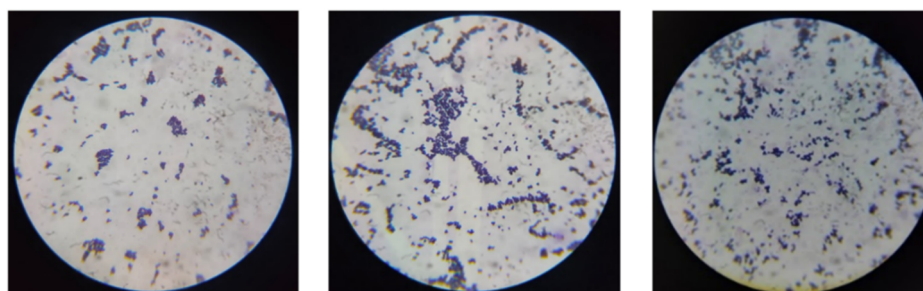


Figure 2. Microscopic observation of Gram-stained bacterial isolates showing Gram-positive characteristics.

3.3. Biochemical Characterisation

Three bacterial isolates, designated PS A, PS B, and PS C, were recovered from poultry-shed soil on Mannitol Salt Agar and were subjected to Gram staining and biochemical characterization. The isolates exhibited Gram-positive cocci arranged in grape-like clusters and showed the biochemical reactions recorded in Table 2. Because individual biochemical reactions may vary among isolates and may not be sufficient for definitive species-level identification, the presumptive identification was further evaluated by PCR amplification of the species-specific *nuc* gene. The detection of the expected *nuc* amplicon provided molecular confirmation of the *S. aureus* isolates (Figure 3).

Table 2. Observed biochemical reactions of the presumptive *Staphylococcus aureus* isolates.

Biochemical Test	PS A	PS B	PS C
Catalase test	Positive (+)	Positive (+)	Positive (+)
Citrate utilization test	Positive (+)	Positive (+)	Positive (+)
Methyl red (MR) test	Positive (+)	Positive (+)	Positive (+)
Voges–Proskauer (VP) test	Negative (–)	Negative (–)	Negative (–)
Oxidase test	Negative (–)	Negative (–)	Negative (–)
Indole test	Negative (–)	Negative (–)	Negative (–)
Urease test	Positive (+)	Negative (–)	Positive (+)

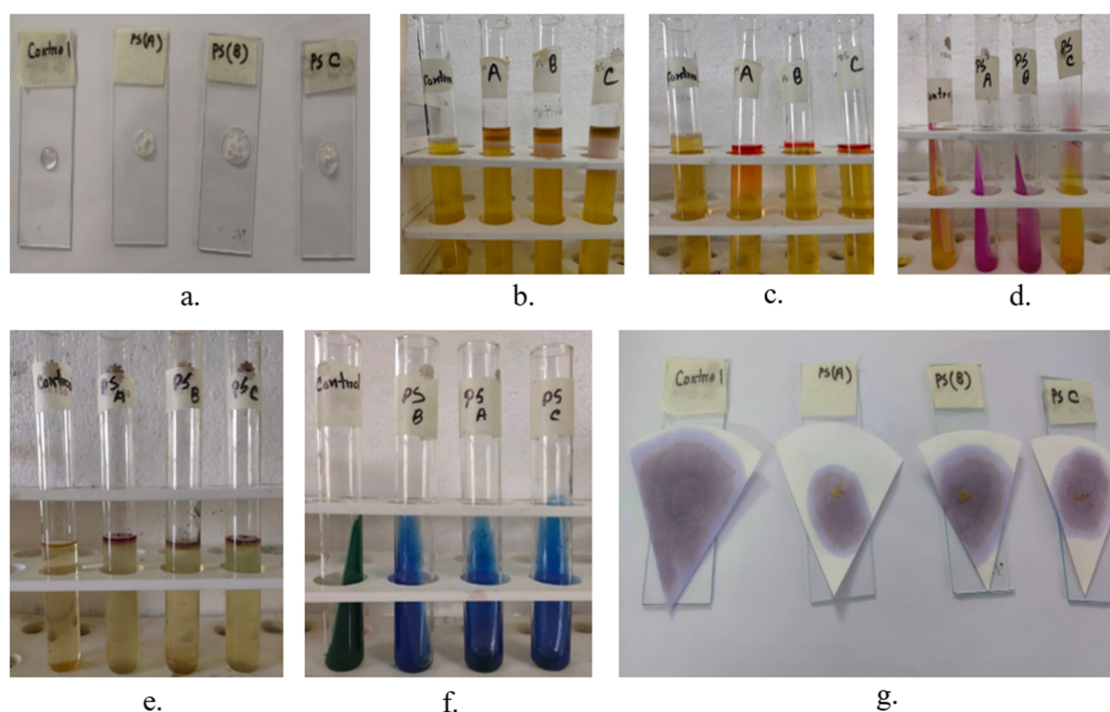


Figure 3. Biochemical characterisation of bacterial isolates: (a) Catalase test, (b) Voges-Proskauer (VP) test, (c) Methyl red test, (d) Urease test, (e) Indole test, (f) Citrate utilisation test, and (g) Oxidase test.

3.4. Molecular Confirmation of *Staphylococcus aureus*

Molecular confirmation of the presumptive *Staphylococcus aureus* isolates was performed by PCR amplification of the species-specific *nuc* gene. Agarose gel electrophoresis revealed amplification products at approximately 278 bp in lanes 1, 2, and 4. No visible amplification products were detected in lanes 3, 5, and 6 or in the no-template negative control (NC). Samples corresponding to lanes 1, 2, and 4 were considered PCR-positive for the *nuc* target under the conditions used (Figure 4).

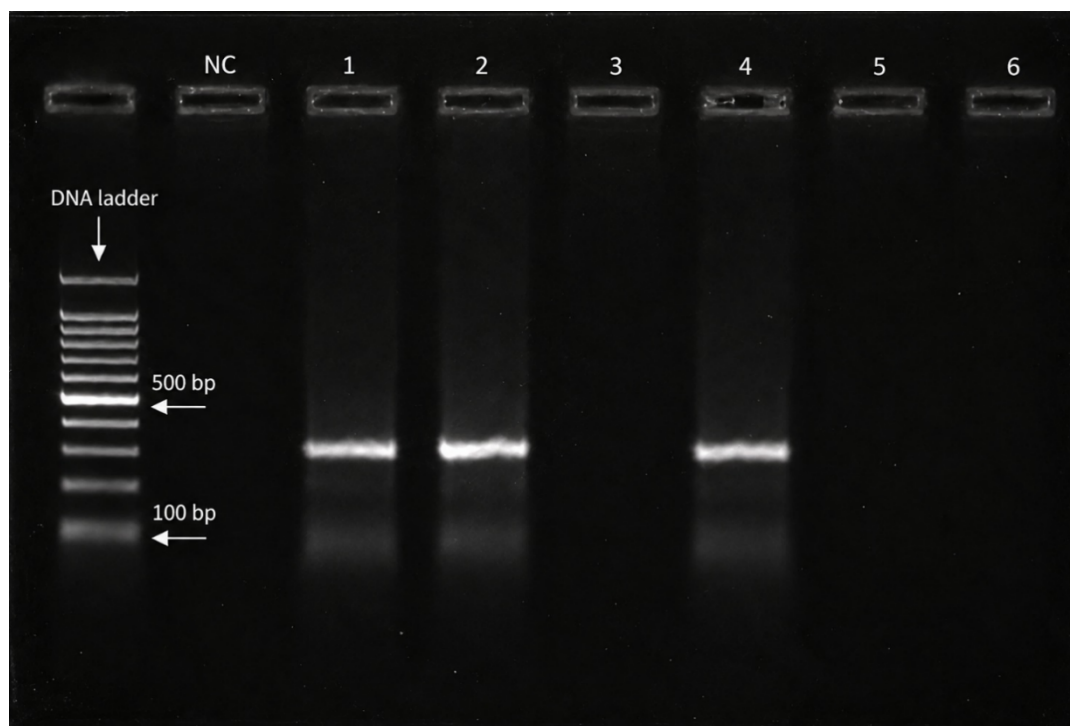


Figure 4. Agarose gel electrophoresis showing PCR amplification of the *Staphylococcus aureus*-specific *nuc* gene.

3.5. Phytochemical Analysis

Qualitative phytochemical screening was conducted to detect the presence of alkaloids, tannins, saponins, flavonoids, coumarins, quinones, phenols, cardiac glycosides, steroids, and triterpenoids in the aqueous, methanolic, and ethanolic extracts of *Moringa oleifera* and *Chenopodium album* (Tables 3 and 4).

Table 3. The results obtained for the qualitative screening of ten phytochemicals of *Moringa oleifera*.

Phytochemicals	Test	Plant Extracts of <i>Moringa oleifera</i>		
		Aqueous	Ethanolic	Methanolic
Alkaloids	Wagner's Reagent	+++	+	+++
Tannins	Ferric Chloride Test	+++	+++	+++
Saponins	Frothing Test	++	++	++
Flavonoids	Lead Acetate Test	–	++	+++
Coumarins	Alkaline Test	–	+++	+++
Quinones	HCL Test	+++	–	+
Phenols	Ferric Chloride Test	+++	+++	+++
Cardiac Glycosides	Keller-Kiliani Test	–	+++	+
Steroids	Salkowski Test	++	+++	–
Triterpenoids	Salkowski Test	–	–	+++

(Key: – = Not present; + = Present in small amounts; ++ = Present in average amount; +++ = Present in significant amount).

Table 4. The results obtained for the qualitative screening of ten phytochemicals of *Chenopodium album*.

Phytochemicals	Test	Plant Extracts of <i>Chenopodium album</i>		
		Aqueous	Ethanolic	Methanolic
Alkaloids	Wagner's Reagent	+++	–	+++
Tannins	Ferric Chloride Test	+++	+++	+++
Saponins	Frothing Test	+++	+++	++
Flavonoids	Lead Acetate Test	+	–	+++
Coumarins	Alkaline Test	++	+++	+++
Quinones	HCL Test	+++	+++	+
Phenols	Ferric Chloride Test	+++	++	+++
Cardiac Glycosides	Keller-Kiliani Test	+++	–	+
Steroids	Salkowski Test	–	–	–
Triterpenoids	Salkowski Test	+	++	+++

(Note: – = Not present; + = Present in small amounts; ++ = Present in average amount; +++ = Present in significant amount).

3.6. Antimicrobial Activity of Plant Extracts

The plant extracts exhibited varying degrees of antibacterial activity against *Staphylococcus aureus*. No measurable inhibition was observed with the aqueous extracts of either *Moringa oleifera* or *Chenopodium album*, whereas the methanolic and ethanolic extracts of both plant species produced measurable inhibition zones. The inhibition-zone measurements are summarized in Table 5. Representative plates showing the antibacterial screening of *M. oleifera* and *C. album* extracts are presented in Figures 5 and 6, respectively, while the comparative presentation of the inhibition-zone diameters is provided in Figure 7. For each extract treatment, the three reported measurements correspond to the three isolates (PS A, PS B, and PS C), and the reported mean $\pm$ SD values were calculated from these three isolate-level observations.

Table 5. Antibacterial activity of plant extracts against *Staphylococcus aureus* expressed as mean zone of inhibition $\pm$ standard deviation (n = 3 independent isolates: PS A, PS B, and PS C).

Plant Species	Extract Type	Zone of Inhibition (Mean $\pm$ SD, mm)	Reference Control (Vancomycin) (mm)
<i>Moringa oleifera</i>	Methanolic extract	34.0 $\pm$ 0.5	22
	Ethanolic extract	20.0 $\pm$ 1.0	22
	Aqueous extract	0.0 $\pm$ 0.0	22
<i>Chenopodium album</i>	Methanolic extract	24.0 $\pm$ 0.6	22
	Ethanolic extract	22.0 $\pm$ 0.4	22
	Aqueous extract	0.0 $\pm$ 0.0	22

Values are expressed as mean $\pm$ standard deviation (SD) based on three isolate-level observations (PS A, PS B, and PS C) per extract treatment (n = 3).

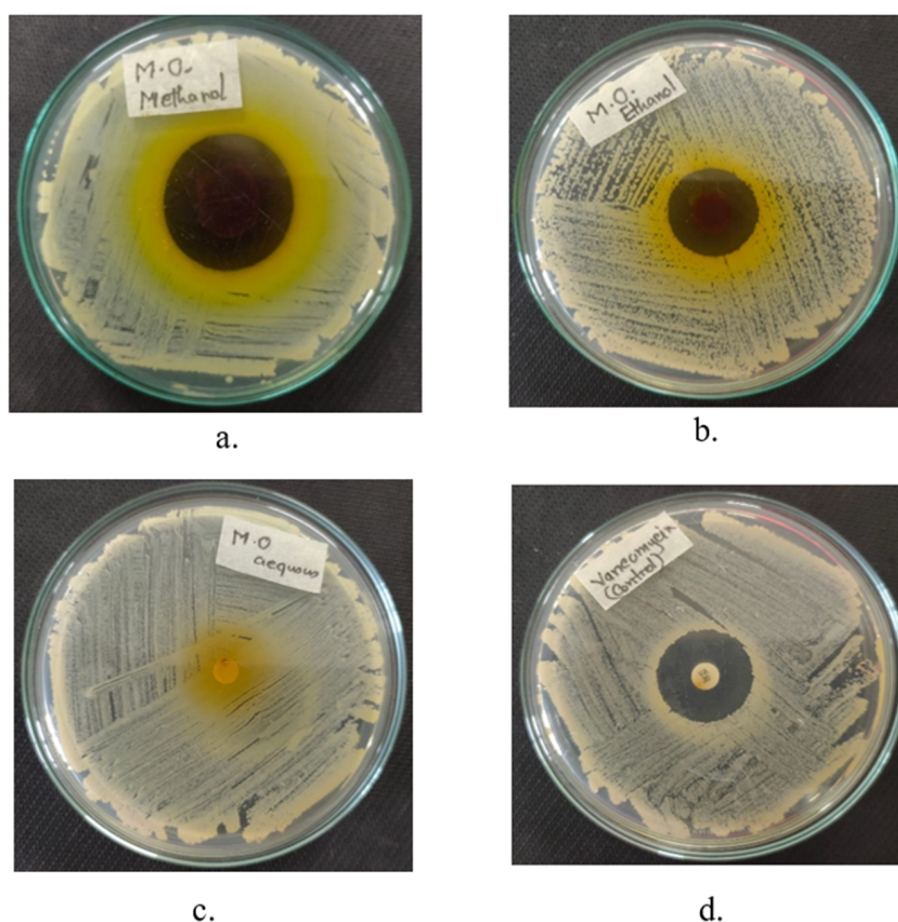


Figure 5. Antimicrobial activity of methanolic, ethanolic, and aqueous extracts of *Moringa oleifera* ((a) Methanolic Extract, (b) Ethanolic Extract, (c) Aqueous Extract, (d) Vancomycin as Control).

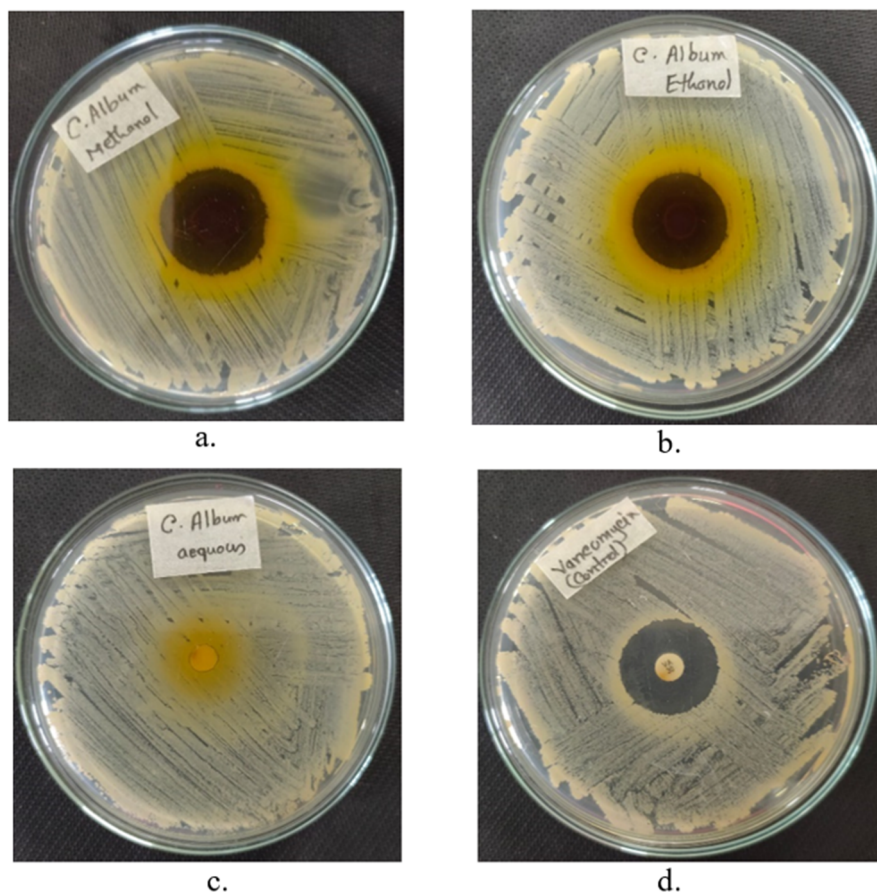


Figure 6. Antimicrobial activity of methanolic, ethanolic, and aqueous extracts of *Chenopodium album* ((a) Methanolic Extract, (b) Ethanolic Extract, (c) Aqueous Extract, (d) Vancomycin as Control).

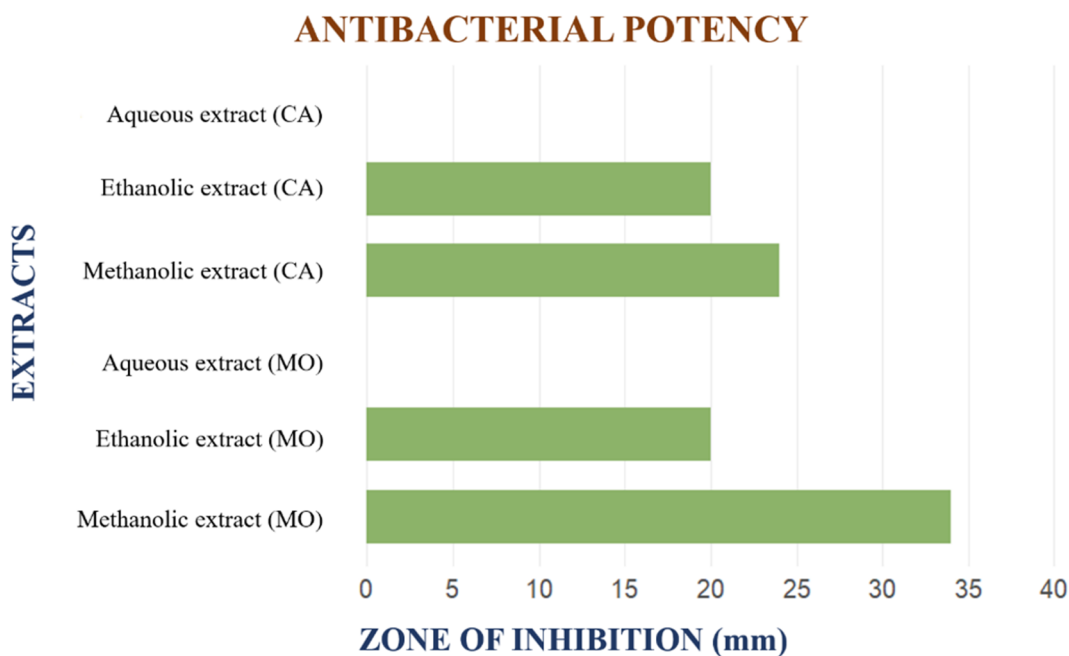


Figure 7. Comparative antibacterial activity of aqueous, ethanolic, and methanolic extracts of *Moringa oleifera* (MO) and *Chenopodium album* (CA) against *Staphylococcus aureus* based on the zone of inhibition (mm).

4. Discussion

The biochemical reactions recorded for the three presumptive isolates (PS A, PS B, and PS C) are presented in Table 2. All three isolates were catalase-positive, citrate-positive, and methyl-red-positive, whereas the Voges-Proskauer (VP), oxidase, and indole tests were negative. Urease activity was positive in PS A and PS C but negative in PS B [24]. These results are reported as observed during the experiments and have not been altered to match an expected biochemical profile. The biochemical tests were used for preliminary characterization rather than as standalone confirmation of species identity. Although the observed reactions provide useful phenotypic information, they do not independently establish the identity of *Staphylococcus aureus*. Therefore, species-level identification must be supported by a validated, species-specific molecular assay and, where available, additional confirmatory tests such as coagulase or DNase testing [25].

Qualitative phytochemical screening revealed the presence of several classes of secondary metabolites in the extracts of *Moringa oleifera* and *Chenopodium album*, including alkaloids, tannins, saponins, flavonoids, coumarins, quinones, phenols, cardiac glycosides, steroids, and triterpenoids. These secondary metabolites have been associated with antimicrobial activity and other biological properties in previous studies [3,26]. The phytochemical profile of *M. oleifera* observed in this study is comparable to that reported by [27,28], which identified alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, cardiac glycosides, coumarins, and quinones in different extracts of the plant. Likewise, the phytochemical composition of *C. album* was generally consistent with previous reports [29].

Molecular confirmation of the presumptive *Staphylococcus aureus* isolates was achieved through amplification of the species-specific *nuc* gene, yielding the expected 278 bp amplicon in PCR-positive isolates. The detection of this target supported molecular confirmation of the presumptive isolates and provided a species-level identification method independent of the variable biochemical reactions. The *nuc* gene, which encodes a thermostable nuclease, has been widely used as a molecular marker for detection and confirmation of *S. aureus*. The findings of the present study are consistent with previous reports demonstrating the utility of *nuc*-based PCR assays for *S. aureus* identification in clinical, food, and environmental samples [15].

The antimicrobial assay demonstrated that both methanolic and ethanolic extracts of *Moringa oleifera* and *Chenopodium album* produced measurable inhibition of *Staphylococcus aureus*, whereas the aqueous extracts showed no detectable inhibition under the experimental conditions. Among the tested plant extracts, the methanolic extract of *M. oleifera* exhibited the largest inhibition zone (34.0 ± 0.5 mm) at a concentration of 10 mg/mL, followed by its ethanolic extract (20.0 ± 1.0 mm). These findings are consistent with previous reports describing the antibacterial activity of alcohol-based *M. oleifera* extracts against *S. aureus* [30]. Similarly, the methanolic and ethanolic extracts of *C. album* produced inhibition zones of 24.0 ± 0.6 mm and 22.0 ± 0.4 mm, respectively, consistent with previously reported antibacterial activity of methanolic *C. album* extracts [31]. The observed inhibition-zone diameters varied with plant species and extraction solvent, highlighting the importance of solvent selection in evaluating the antibacterial properties of plant extracts. Future investigations incorporating matched solvent-only controls for methanol, ethanol, and distilled water would help distinguish extract-associated antibacterial effects from potential contributions of the reconstitution solvents.

The larger inhibition zones observed with the methanolic extracts suggest that methanol may facilitate the extraction of constituents associated with antibacterial activity. The qualitative phytochemical screening identified several classes of secondary metabolites in both plant species, providing a basis for further investigation of their potential contribution to the observed antibacterial effects. Quantitative phytochemical profiling, bioactivity-guided fractionation, and chemical characterisation could further clarify the relationship between extract composition and antibacterial activity. Although the methanolic extract of *M. oleifera* produced an inhibition zone of 34.0 ± 0.5 mm, compared with 22 mm for the vancomycin reference control, this difference in inhibition-zone diameter does not establish greater antibacterial potency than vancomycin. Crude plant extracts and purified antibiotics differ in composition, concentration basis, and diffusion behaviour in agar; therefore, direct potency comparisons require appropriately standardized quantitative assessments.

Overall, the findings provide preliminary *in vitro* evidence that *Moringa oleifera* and *Chenopodium album* leaf extracts can inhibit the tested *Staphylococcus aureus* under the assay conditions used. Methanolic extracts produced the largest inhibition zones among the plant extracts tested. Qualitative phytochemical screening indicated the presence of several classes of secondary metabolites; however, the present analysis does not establish which individual compounds were responsible for the observed antibacterial effects. Quantitative chemical analysis and isolation or direct identification of active constituents would be required to determine their specific contributions. These findings therefore support further investigation of the extracts using quantitative phytochemical analysis, MIC and MBC determination, broader isolate panels, and identification of active constituents.

Limitations of the Study

Several limitations should be considered when interpreting the findings of this study. First, the plant materials were evaluated as crude extracts, and the individual compounds responsible for the observed antibacterial activity were neither isolated nor chemically characterized. Second, phytochemical screening was qualitative and therefore did not determine the concentrations of the detected constituents. Third, antibacterial activity was assessed using the disc-diffusion assay, without determination of the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC). Consequently, the findings do not establish the minimum concentrations required to inhibit or kill the tested bacteria. Fourth, the study involved only three *Staphylococcus aureus* isolates, limiting the generalizability of the findings to broader *S. aureus* populations. Fifth, antibiotic susceptibility testing was not performed; therefore, the isolates could not be classified as antibiotic-resistant or antibiotic-susceptible on the basis of this study. In addition, solvent-only negative controls were not included in the original assay, so the potential contribution of methanol, ethanol, and distilled water to the observed inhibition zones could not be independently assessed. Finally, each extract was tested once against each isolate, without additional technical replicate discs or repeated experimental runs, limiting the assessment of experimental repeatability. Future studies should incorporate matched solvent-only controls, a larger and more diverse panel of bacterial isolates, antibiotic susceptibility testing where resistance status is relevant, MIC and MBC determination, quantitative phytochemical analysis, and the isolation and chemical characterization of potentially active compounds. Repeated antibacterial assays would also help establish the reproducibility of the observed effects.

5. Conclusions

The present study demonstrated that methanolic and ethanolic leaf extracts of *Moringa oleifera* and *Chenopodium album* exhibited antibacterial activity against the tested *Staphylococcus aureus* isolates, whereas the aqueous extracts showed no detectable inhibition under the experimental conditions. Among the plant extracts, the methanolic extract of *M. oleifera* produced the largest inhibition zone. The qualitative phytochemical screening indicated the presence of several classes of secondary metabolites that may contribute to the observed activity; however, the specific compounds responsible were not determined. These findings provide preliminary *in vitro* evidence supporting further investigation of these plant extracts as potential sources of antibacterial compounds. Additional studies involving antibiotic susceptibility testing where applicable, MIC and MBC determination, quantitative phytochemical characterization, identification of active constituents, broader isolate panels, and appropriate safety and efficacy assessments are required before any therapeutic application can be considered.

Author Contributions: I.J.B. contributed to conceptualization, investigation, methodology, data curation, formal analysis, validation, visualization, writing the original draft, and writing—review and editing. S.B.S. contributed to formal analysis, project administration, supervision, and validation. F.A.N. contributed to conceptualization, investigation, methodology, data curation, formal analysis, visualization, writing the original draft, and writing—review and editing. A.D. contributed to conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing the original draft, and writing—review and editing. M.M.H.K. contributed to formal analysis, funding acquisition, project administration, supervision, validation, and writing—review and editing. S.N. contributed to investigation, methodology, and data curation. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical approval was not required for this study, as it did not involve human participants or live animals. Soil samples were collected from poultry shed environments, and all procedures complied with relevant institutional and national guidelines.

Informed Consent Statement: Not applicable.

Data Availability Statement: The corresponding author can provide the datasets generated and analyzed during this study upon reasonable request.

Acknowledgments: The authors sincerely thank the Faculty of Biotechnology and Genetic Engineering, Sylhet Agricultural University, for providing laboratory facilities and technical support to carry out this research work.

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

Use of AI and AI-Assisted Technologies: During the preparation of this work, the authors acknowledge the use of OpenAI for minor assistance with English-language editing and grammatical refinement of the manuscript. The authors take full responsibility for the content of the published article.

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