Journal of Applied Biology & Biotechnology Vol. 14(6), pp. 96-106, November-December, 2026
Available online at http://www.jabonline.in
DOI: 10.7324/jabb.2026.314800

Agro-waste valorization of sweet potato peel waste for production and characterization of antibacterial bioactive metabolites from *Streptomyces diastaticus*

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ARTICLE INFO

Article history:
Received on: 23/03/2026
Accepted on: 04/07/2026
Available Online: 25/09/2026

Key words:
Bioactive metabolites, agro-waste valorization, antibacterial activity, antimicrobial resistance, *Streptomyces diastaticus*, sweet potato peel waste

ABSTRACT

The emergence of antimicrobial resistance (AMR) in clinical, environmental, and agricultural settings is a major global concern, necessitating sustainable and environmentally friendly solutions. Simultaneously, environmental pollution resulting from the improper disposal of agro-wastes poses an additional challenge requiring effective waste management strategies. A soil isolate identified as *Streptomyces diastaticus* (GenBank accession number PQ469050.1) was utilized for the production of bioactive metabolites. Apple pomace, sweet potato peel, and rice husk were evaluated as agro-waste substrates, and sweet potato peel waste was selected on the basis of the highest crude metabolite yield obtained after solvent extraction. Fermentation was carried out using the selected substrate, and the resulting fermentation broth was extracted with ethyl acetate. The crude extract was subsequently subjected to partial purification by column chromatography, followed by characterization using Fourier transform infrared spectroscopy, Gas Chromatography–Mass Spectrometry, and ^{1}H Nuclear Magnetic Resonance. The antibacterial activity of the partially purified bioactive fraction was evaluated against selected human pathogens: *Escherichia coli* (ATCC 4157), *Klebsiella pneumoniae* (ATCC 13882), and *Shigella flexneri* (ATCC 9199). The highest antibacterial activity was observed against *K. pneumoniae*, with a zone of inhibition of 17 mm. These findings demonstrate that sweet potato peel waste can serve as an effective and low-cost substrate for the production of bioactive metabolites by *S. diastaticus*, contributing to agro-waste valorization and providing a basis for the development of potential antimicrobial agents.

1. INTRODUCTION

The accumulation of agro-wastes in the environment is currently becoming a growing concern in many parts of the world. The problem is largely caused by a lack of adequate strategies and sustainable options for their proper disposal. Although most of the agro-wastes are utilized as animal feed or incorporated into food additives, there is still a significant amount of waste that ends up in landfills [1]. Therefore, they lead to environmental pollution and can sometimes serve as breeding grounds for pathogens [2,3].

Meanwhile, antimicrobial resistance (AMR) has been declared by the World Health Organization as one of the major global threats to public health [4]. It has contributed to increased morbidity, mortality, and challenges in the effective management of infectious diseases [5,6]. The reduced efficacy of existing antibiotics has complicated the treatment of infections and increased the risk associated with medical procedures such as surgery [7]. In response to these challenges, there is a growing emphasis on the discovery of novel antimicrobial agents that are not only effective, but also environmentally sustainable. Natural sources, particularly microorganisms, have historically played an important role in antibiotic discovery and continue to offer potential opportunities for the development of new bioactive metabolites. Addressing this challenge requires coordinated efforts from various sectors [8–10].

As the search for effective antimicrobials continues, researchers are increasingly focused on identifying sustainable sources of bioactive compounds to address AMR. In this context, biological sources of novel antimicrobial agents have become a major research priority [11–13].

Members of the genus *Streptomyces,* which belong to the phylum Actinobacteria, have received considerable research attention [14,15] due to their ability to produce a wide range of secondary metabolites with diverse therapeutic applications. These metabolites include antibiotics, antifungals, and anticancer compounds, and *Streptomyces* species are responsible for more than half of the clinically used antibiotics [16]. Owing to their remarkable capacity to produce

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bioactive compounds, *Streptomyces* remain a promising source of novel antimicrobial compounds.

In addition to exploring microbial sources, recent studies have highlighted the importance of using agro-wastes in microbial fermentation processes [3]. They offer significant advantages as a fermentation substrate due to their abundance, low costs, and can be utilized by microbes for their growth and metabolite production. Most microorganisms, including *Streptomyces,* can break down these wastes and use them as nutritional sources. Several studies have demonstrated the potential of agro-wastes as an economical and sustainable alternative to conventional culture media for microbial fermentation. Various agro-industrial residues including coconut oil cake, sugarcane bagasse, groundnut shell, fruit pomace, rice bran, and other agricultural by-products have been successfully employed as substrates for production of enzymes, pigments, antibiotics, and biopolymers by microorganisms [17,18]. The utilization of such residues not only reduces production costs, but also contributes to waste valorization and environmental sustainability.

Therefore, due to the high costs that are associated with the production and development of new antibiotics, and a global need for environmentally sustainable solutions, this study aimed to produce bioactive metabolites from *Streptomyces diastaticus* using sweet potato peel waste and evaluate their antibacterial activity against human pathogens. Sweet potato peel waste is an abundant agro-residue generated during food processing and household consumption. It contains substantial amounts of carbohydrates, starch, simple sugars, dietary fiber, minerals, vitamins, and other nutrients that can support microbial growth and metabolite production. Owing to its nutrient-rich composition, widespread availability, and underutilization, sweet potato peel waste was selected as the substrate in the present study to evaluate its potential for sustainable bioactive metabolite production by *S. diastaticus* [19].

The present study demonstrates the potential of utilizing agro-wastes for the dual purpose of reducing environmental pollution through waste valorization while contributing to the discovery of bioactive metabolites with antibacterial potential that may help to address the global challenge of AMR.

2. MATERIALS AND METHODS

2.1. Soil Sample Collection and Isolation of *Streptomyces* sp.

Isolation was done using soil samples collected from the Maruthamalai hills forest area in Coimbatore, Tamil Nadu, India, following standard procedures [20]. Samples were collected by employing aseptic techniques, including the use of sterile spatulas and zipped bags. During sample collection, the top layer of soil was carefully removed, and samples were obtained at a depth between 10 and 15 cm. They were immediately stored at 4°C until further processing.

A serial dilution of up to 10^{-6} was performed by dissolving 1 g of soil into 10 ml of sterile distilled water. The mixture was vigorously shaken, and 0.1 ml of each dilution was poured and spread properly on Starch Casein Nitrate Agar (SCA) plates, which are composed of soluble starch: 10 g, K_2HPO_4: 2 g, NaCl: 2 g, KNO_3: 2 g, casein: 0.3 g, $MgSO_4.7H_2O$: 0.05 g, $CaCO_3$: 0.02 g, $FeSO_4.7H_2O$: 0.01 g, agar: 15 g, and distilled water: 1,000 ml, and final pH was adjusted to 7.0. The inoculated plates were incubated at 37°C for 7 days.

Isolated colonies were selected and purified on SCA and stored at 4°C for future use [21].

2.2. Identification of *Streptomyces sp.*

The isolate EMB BAC 24 was morphologically characterized following the procedures described by Shirling and Gottlieb [22] and subjected to Gram staining. To confirm the taxonomic identity of the isolate, including its genus and species, 16S rRNA gene sequencing was performed.

2.3. Molecular Identification by 16S rRNA Gene Sequencing

Genomic DNA was isolated from EMB BAC 24, and the quality of DNA was assessed by 1% agarose gel electrophoresis. The 16S rRNA gene was amplified using the actinomycete-specific primers ACT_F243 (5′-GGATGAGCCCGCGGCCTA-3′) and ACT_R1378 (5′-CGGTGTGTACAAGGCCCGGGAACG-3′) [23]. Polymerase chain reaction (PCR) amplification was carried out in 25 µl reaction mixture containing 12 µl of 2×Taq Master Mix (containing Taq DNA polymerase, 0.4 mM dNTPs, and 3.2 mM $MgCl_2$), 1.5 µl of each forward and reverse primers, 5 µl of template DNA, and 5 µl of deionized water. PCR conditions consisted of an initial denaturation at 95ºC for 3 minutes, then 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, an extension at 72ºC for 45 seconds, with a final extension at 72ºC for 3 minutes. The amplified PCR product was purified using QIAquick PCR Purification Kit (QIAGEN, Germany) and subjected to sequencing. Forward and reverse sequencing reactions were performed using the same primers, and sequencing was carried out using BigDye™ and Terminator v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. The obtained sequence was compared with the sequences in the NCBI database using BLAST, and phylogenetic analysis was performed using MEGA11 software to determine the isolate's taxonomic position [24]. The 16S rRNA gene sequence was submitted to GenBank and was assigned an accession number.

2.4. Optimization of Temperature, pH, and Salinity

The growth of the isolate EMB BAC 24 was observed under different pH, temperature, and salinity conditions following standard physiological characterization procedures for *Streptomyces* species [25]. pH was adjusted from 5 to 9 by using 1N of HCl and 1N of NaOH, and salt concentration from 1% to 5% was regulated by using NaCl. The growth of isolate was observed at 37°C, 40°C, 45°C, and 50°C. A loopful of culture was inoculated into nutrient broth and incubated at 37°C with shaking at 150 rpm for 33 hours. Growth under each condition was monitored by measuring the optical density at 600 nm (OD_{600}) using a UV–Visible spectrophotometer [26].

2.5. Preparation of Agro-Wastes and Production of Bioactive Metabolites

Agricultural waste substrates, including apple pomace, sweet potato peel, and rice husk, were collected, cleaned, shade-dried, and ground into fine particles using a laboratory grinder and stored in airtight containers until further use. About 3 g of the prepared agro-waste substrate was soaked in 180 ml of hot water overnight to facilitate the release of soluble nutrients from the substrate [18]. About 20 ml of inoculum prepared in Starch Casein Broth (SCB) was added to sterile pretreated broth to obtain a final volume of 200 ml. This was used as the growth medium for EMB BAC 24 for bioactive metabolite production. The experiment was performed in triplicate, with SCB serving as the control. Incubation was carried out at 37°C under shaking conditions (150 rpm), and the growth of the isolate was monitored for 12 days by measuring the optical density (OD600) at 600 nm using a UV–Visible spectrophotometer.

2.6. Optimization of Carbon and Nitrogen Sources

Three carbon sources (glucose, sucrose, and maltose) and nitrogen sources (yeast extract, peptone, and urea) were selected to determine the most suitable source for the growth of the isolate EMB BAC 24 following standard optimization procedures [27]. The isolate was grown in 3% concentrations of each nutrient source, and its growth was monitored for 12 days. All optimization experiments were conducted using sweet potato peel broth in triplicate. Sweet potato peel broth without carbon and nitrogen sources served as a control.

2.7. Solvent Extraction and Partial Purification of the Bioactive Fraction

Fermentation broth was centrifuged at 5,000 rpm for 15 minutes to separate the biomass. The resulting cell-free supernatant was mixed with ethyl acetate in a 1:1 (v/v) ratio. The mixture was thoroughly shaken and transferred to a separating funnel, where it was allowed to settle for phase separation. The organic solvent layer was then collected into an evaporating dish and allowed to evaporate completely at room temperature. The dried crude extract was then weighed using an analytical balance, and crude metabolite yield was expressed as g/l of fermentation broth. All experiments were performed in triplicate. The resulting crude extract was subjected to further purification using chromatographic techniques. Partial purification of the bioactive fraction was carried out using column chromatography as reported in previous studies with slight modifications [28]. In the present study, a glass column (15 × 300 mm) was packed with silica gel prepared as slurry in ethyl acetate. After uniform and compact packing of the silica gel, a layer of clean, dried sand was added on top to stabilize the stationary phase. Elution was performed using a stepwise solvent gradient of ethyl acetate–methanol (8:2, 6:4, and 5:5, v/v) to facilitate efficient separation of the bioactive metabolites. Fractions were collected in 5 ml test tubes and used for antibacterial activity assay.

2.8. Characterization of the Partially Purified Bioactive Fraction

The partially purified bioactive fraction was characterized by Fourier Transform Infrared (FT-IR) spectroscopy to identify the functional groups present in the bioactive fraction obtained from EMB BAC 24. Furthermore, gas chromatography–mass spectrometry (GC–MS) was employed to putatively identify the bioactive metabolites present in the partially purified bioactive fraction based on their mass-to-charge ratio by comparison with the National Institute of Standards and Technology (NIST) Mass Spectral Library. In addition, Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy was performed to characterize the structural features of the partially purified bioactive fraction by analyzing the types of hydrogen present [29,30].

2.9. Antibacterial Activity of the Partially Purified Bioactive Fraction

Mueller–Hinton Agar (MHA) was used for the agar well diffusion assay. A 24 hour-old culture of the three bacterial test pathogens, *Escherichia coli* (ATCC 4157), *Klebsiella pneumoniae* (ATCC 13882), and *Shigella flexneri* (ATCC 9199), was swabbed onto the surface of individual MHA plates using sterile cotton swabs. The plates were left at room temperature for 20 minutes to allow the bacterial suspension to diffuse into the medium. Wells of 6 mm diameter were made in the agar using sterile micropipette tips. Aliquots (100 µl) containing 20, 40, or 60 µg of the partially purified bioactive fraction dissolved in Dimethyl Sulfoxide (DMSO) were dispensed into the wells, providing doses of 20, 40, and 60 µg per well, respectively. DMSO served as the negative control. Commercially available antibiotic discs (HiMedia Laboratories Pvt. Ltd., Mumbai, India), including gentamicin (10 µg), tetracycline (30 µg), ampicillin (10 µg), and kanamycin (30 µg), were included as positive controls for reference. The antibiotic disc potencies were consistent with those recommended by the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing [31]. The plates were incubated at 37°C for 24 hours. After incubation, antibacterial activity was evaluated by measuring the zones of inhibition.

2.10. Statistical Analysis

All experiments in this study were conducted in triplicate. The means were calculated, and standard deviations were determined using Origin Pro graphing and analysis program version 2021. The results presented in the bar graph include the standard error bars.

3. RESULTS AND DISCUSSION

3.1. Isolation and Morphological Characteristics of *S. diastaticus*

After the incubation period, the isolated colonies on the spread plates were examined. The isolate formed dry, powdery colonies with reddish-pink pigmentation on SCA. Upon purification, the isolate exhibited well-developed white aerial mycelium with pinkish-red substrate mycelium that diffused into the medium. The isolate EMB BAC 24 exhibited morphological characteristics typical of the genus *Streptomyces* and was found to be Gram-positive. These characteristics are consistent with those commonly reported for members of the genus *Streptomyces*. Pigment production in *Streptomyces* species has been associated with the biosynthesis of secondary metabolites [32,33]. Based on its characteristic *Streptomyces*-like morphology and Gram-positive nature, the isolate was selected for further investigation of its antibacterial potential.

3.2. Identification of *Streptomyces* isolate by 16S rRNA Gene Sequencing

The isolate was identified at the molecular level using partial 16S rRNA gene sequencing, and the sequence was deposited in GenBank under accession number PQ469050.1. PCR amplification produced a single discrete amplicon of approximately 1,100 bp (Fig. 1). BLAST analysis of the partial 16S rRNA gene sequence revealed 100% query coverage, 100% sequence identity, and an *E*-value of 0.0 with several *S. diastaticus* sequences deposited in GenBank, including strains MN422355.1, MW217181.1, and MW217079.1. Phylogenetic analysis based on the partial 16S rRNA gene sequence further supported the placement of isolate EMB BAC 24 within the *S. diastaticus* lineage (Fig. 2). The phylogenetic tree was constructed using the neighbor joining method with 1,000 bootstrap replicates in MEGA11. Similar molecular approaches have been employed for the identification of *Streptomyces* isolates [34]. Although the partial 16S rRNA gene sequence provided strong support for the taxonomic assignment of the isolate, multilocus sequence analysis or genome-based approaches may provide higher species level resolution for closely related *Streptomyces* species.

The recovery of *S. diastaticus* from Maruthamalai hills forest soil expands the range of ecological niches explored for bioactive *Streptomyces* and highlights the potential of forest soil ecosystems as reservoirs of antibiotic-producing *Streptomyces*. Previous studies have reported *S. diastaticus* strains from diverse terrestrial environments, including the Western Ghats and rhizosphere soils [33,35,36]. The present isolate was recovered from Maruthamalai hills forest soil,

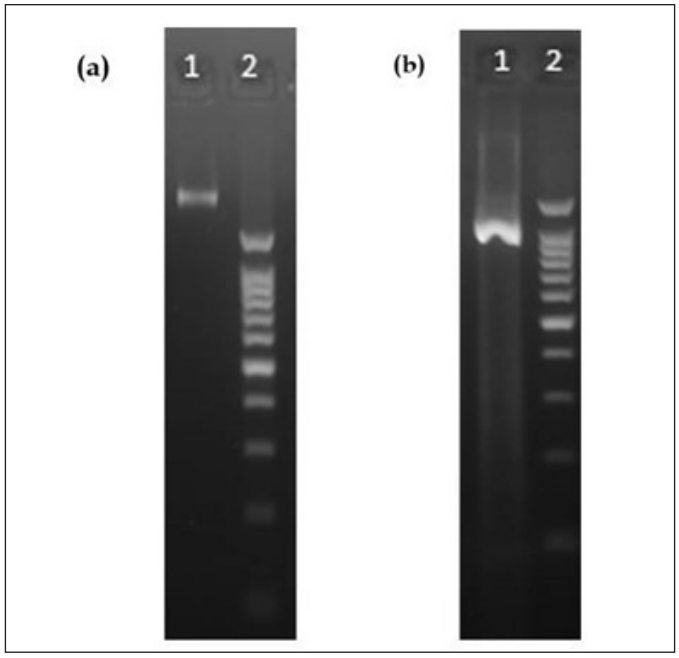


Figure 1. Agarose gel electrophoresis of *S. diastaticus* (a) Genomic DNA. (b) PCR-amplified 16S rRNA gene showing a single amplicon of approximately 1,100 bp.

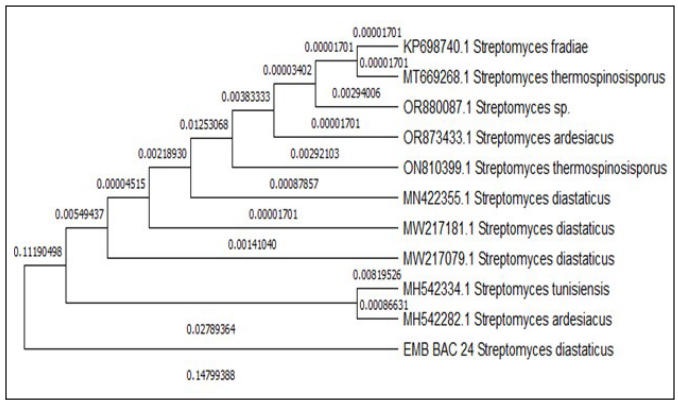


Figure 2. Phylogenetic tree based on the 16S rRNA gene sequence of *S. diastaticus.*

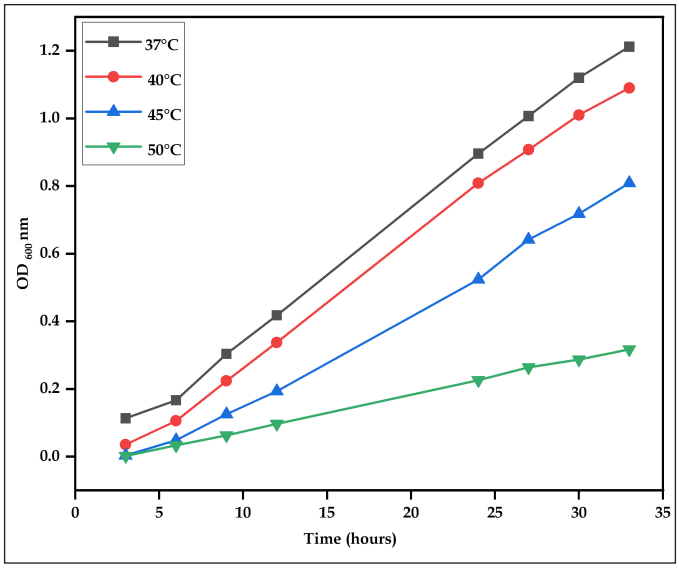


Figure 3. Effect of temperature on the growth of *S. diastaticus.*

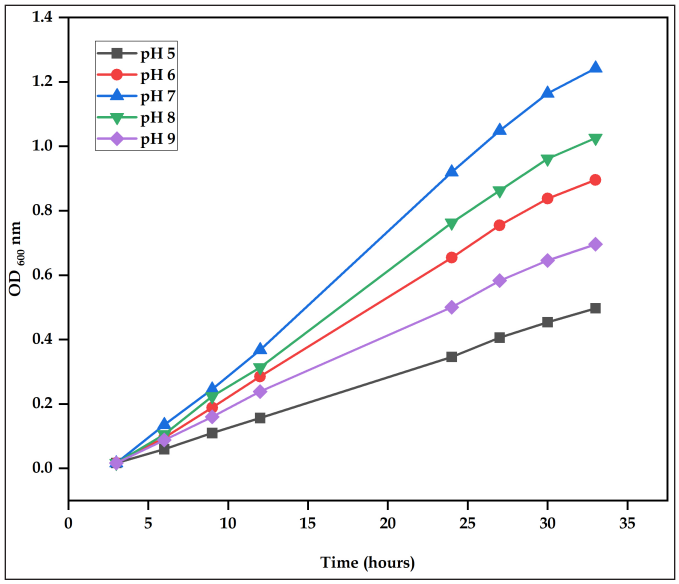


Figure 4. Effect of pH on the growth of *S. diastaticus.*

suggesting that diverse terrestrial habitats may harbor *Streptomyces* with potential for bioactive metabolite production. Furthermore, studies from India and other regions have reported the ability of *Streptomyces* species to produce bioactive metabolites using agro-waste substrates [37,38]. However, reports describing antibiotic production by *S. diastaticus* utilizing sweet potato peel waste remain limited.

3.3. Effect of Temperature, pH, and Salinity on the Growth of *S. diastaticus*

The growth of *S. diastaticus* was highest at 37°C and progressively declined as the temperature increased to 50°C (Fig. 3). The observed higher growth at 37°C compared with other temperatures tested may be attributed to enhanced enzymatic activity and metabolic efficiency under these conditions. The decline in growth under higher temperature may be due to the reduced physiological performance and thermal stress. Temperature is a key environmental factor influencing microbial growth and secondary metabolite production in *Streptomyces* species [39].

In addition to temperature, pH was also found to influence the growth of the isolate. The growth was highest within the pH range of 7–8 (Fig. 4), whereas reduced growth was observed under acidic conditions and at pH 9. The decline in growth at extreme pH values may be due to their adverse effects on enzyme activity, nutrient uptake, and cellular physiology [40]. The optimal growth observed at pH 7–8 is consistent with previous studies reporting that most *Streptomyces* spp. grow well under neutral to slightly alkaline conditions [41], suggesting that *S. diastaticus* possesses physiological characteristics typical of members of this genus.

Under saline conditions, its growth was highest at 1% NaCl and progressively decreased with increasing salt content (Fig. 5). The

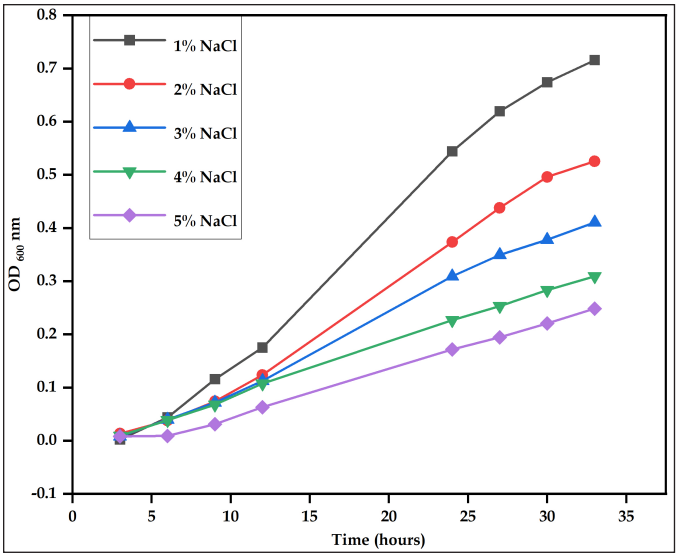


Figure 5. Effect of salinity on the growth of *S. diastaticus.*

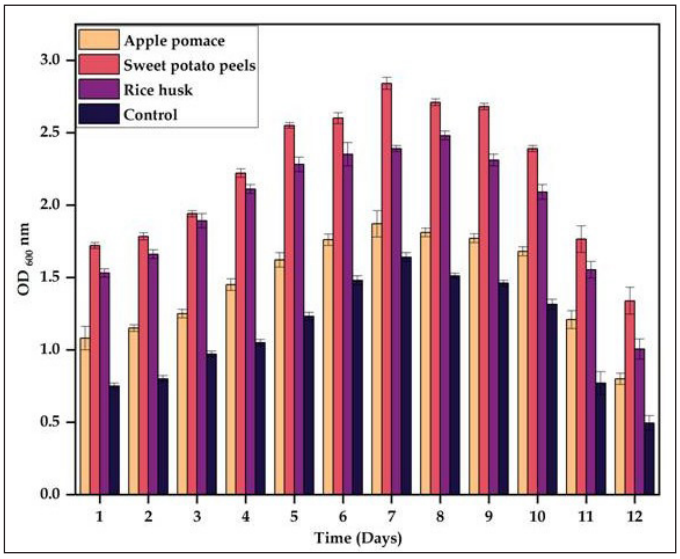


Figure 6. Growth profile of *S. diastaticus* using different agro-waste substrates.

ability of the isolate to grow across the tested salinity range suggests moderate salt tolerance. However, higher salt concentrations may create osmotic stress, which in turn can inhibit growth and affect the synthesis of secondary metabolites. Similar observations have been reported in previous studies where *Streptomyces spp.* showed the ability to withstand varying salt concentrations. Unlike *Streptomyces radiopugnans,* which reportedly exhibits optimal growth at 3% NaCl, *S. diastaticus* showed maximum growth at 1% NaCl, indicating a lower salinity requirement. Other studies have documented a decline in *Streptomyces* growth with increasing salt concentration [42,43].

3.4. Production of Bioactive Metabolites Using Sweet Potato Peel Waste

The growth of *S. diastaticus* was monitored in apple pomace, sweet potato peel waste, and rice husk over a 12-day incubation period (Fig. 6). The highest crude metabolite yield was obtained from sweet potato peel waste (1.521 ± 0.094 g/l), followed by apple pomace (1.311 ± 0.077 g/l), rice husk (1.132 ± 0.088 g/l), and the control (0.972 ± 0.065 g/l). Furthermore, its growth in all three agro-wastes was higher than that observed in SCB, which was the control in this experiment. Apple pomace, sweet potato peel, and rice husk were selected because they are abundant agricultural residues generated from fruit processing, root crop consumption, and rice production, respectively. These wastes differ considerably in their nutritional composition, ranging from sugar–starch-rich substrates (apple pomace and sweet potato peel) to high lignocellulosic content, making them suitable candidates for evaluating the ability of *S. diastaticus* to utilize diverse agro-waste resources for growth and metabolite production.

The higher yield obtained with sweet potato peel waste suggests that it provided a more favorable nutritional source, supplying readily utilizable carbon and other nutrients required for enhanced metabolite production [19]. These nutrients may have contributed to enhanced growth and metabolite production observed in *S. diastaticus*. In contrast, rice husk consists predominantly of lignocellulosic components, including cellulose, hemicellulose, and lignin, which require enzymatic degradation before their utilization, thereby potentially limiting microbial growth and metabolite synthesis. Although apple pomace is rich in sugars, organic acids, and phenolic compounds, it supported lower growth than sweet potato peel, possibly due to rapid depletion of readily available sugars and the presence of inhibitory compounds. Similar observations have been reported in previous studies, where agro-waste substrates supported higher metabolite production than conventional synthetic media [18,44].

The ability of the isolate to grow in these agro-wastes highlights its potential as a sustainable and cost-effective substrate for the fermentation process. The utilization of these wastes by *S. diastaticus* enhanced its growth and may contribute to improved metabolite production.

3.5. Growth of *S. diastaticus* Using Different Carbon and Nitrogen Sources

The availability of carbon and nitrogen sources plays a critical role in microbial growth and metabolism. In the present study, the growth of *S. diastaticus* was evaluated using different carbon and nitrogen sources. Among the carbon sources used, glucose supported the highest growth compared to maltose, sucrose, and the control (Fig. 7). These findings may be attributed to the fact that glucose is a simple sugar, and therefore, it can easily be assimilated by the isolate, thereby causing rapid growth and biomass accumulation. Nitrogen sources also influenced the growth of the isolate. As shown in Figure 8, the highest growth was observed in peptone as compared to yeast extract, urea, and the control. The highest growth observed in peptone may be attributed to its rich content of amino acids and peptides, which are essential for cellular growth and metabolic activities. The study's findings indicate that glucose and peptone are favorable carbon and nitrogen sources for the optimal growth of *S. diastaticus*. These observations are in line with previous studies, which demonstrated the important role of carbon and nitrogen sources in regulating growth and secondary metabolite production in *Streptomyces* species [16,45]. Therefore, optimizing nutrient sources is important for enhancing the growth of *S. diastaticus* and may contribute to improved production of bioactive secondary metabolites.

3.6. Purification of the Partially Purified Bioactive Fraction

Partial purification of the bioactive fraction extracted from *S. diastaticus* resulted in a total of 14 fractions. Among these, five

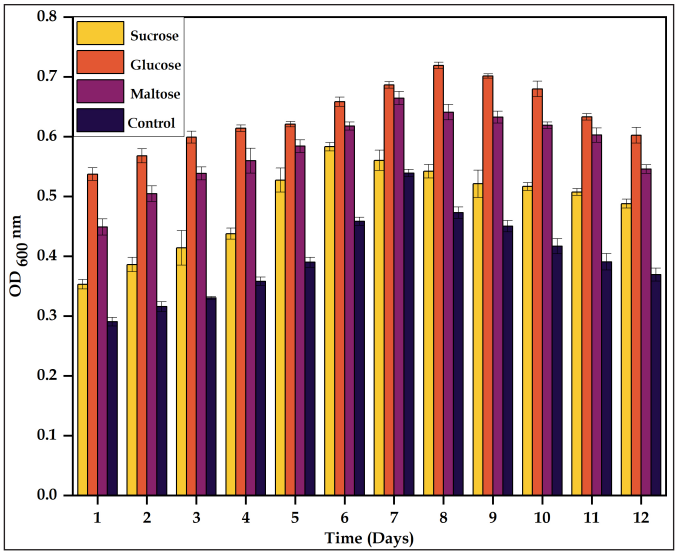


Figure 7. Growth profile of *S. diastaticus* using different carbon sources.

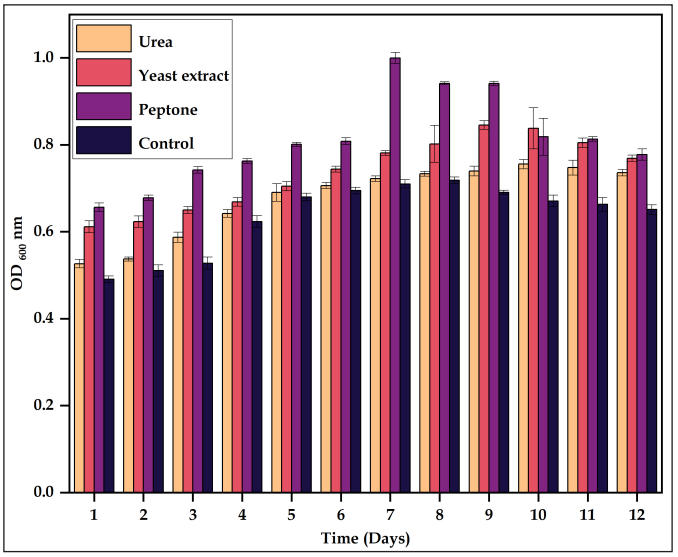


Figure 8. Growth profile of *S. diastaticus* using different nitrogen sources.

fractions exhibited antibacterial activity against the tested bacterial pathogens. The observed antibacterial activity across multiple fractions suggests that the crude extract contained bioactive metabolites that were separated during fractionation. Among the active fractions, the fraction exhibiting the largest inhibition zone was considered the most active and was selected for further characterization. FTIR, GC–MS, and ^{1}H-NMR analyses were performed to characterize the functional groups and putatively identify the bioactive metabolites associated with the observed antibacterial activity.

3.7. Characterization of the Partially Purified Bioactive Fraction

3.7.1. Fourier transform infrared spectroscopy

The partially purified bioactive fraction was subjected to FTIR analysis to identify the functional groups present in the bioactive fraction. The FTIR spectrum (Fig. 9) exhibited characteristic absorption bands

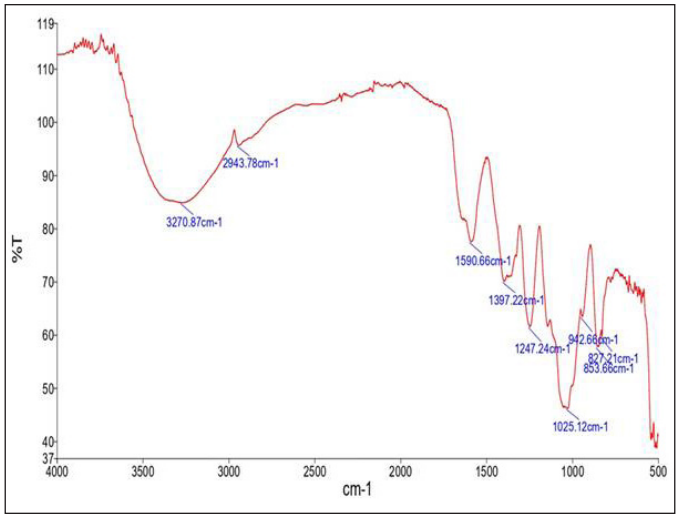


Figure 9. FTIR spectrum of the partially purified bioactive fraction extracted from *S. diastaticus.*

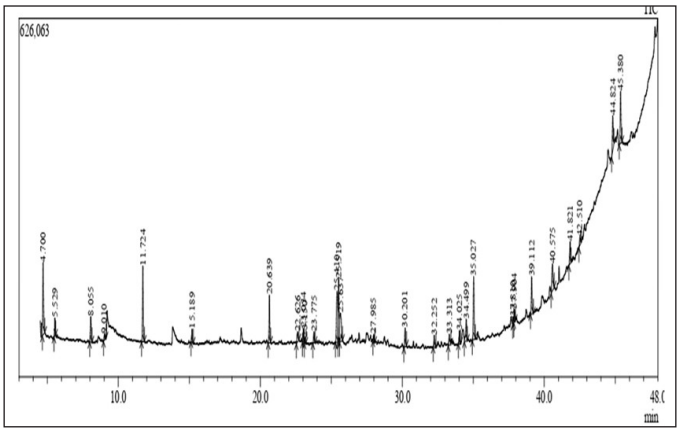


Figure 10. Gas chromatogram of partially purified bioactive fraction extracted from *S. diastaticus.*

corresponding to different functional groups. The broad absorption band at 3,270.87 cm^{-1} was assigned to O–H stretching, while the band at 1,397.22 cm^{-1} was attributed to O–H bending vibrations, suggesting the presence of hydroxyl-containing compounds such as alcohols, phenols, or carboxylic acids. These functional groups have been reported in several bioactive metabolites with antimicrobial properties due to their ability to interact with microbial cell membranes. The absorption bands observed at 1,247.24 and 1,025.12 cm^{-1} indicate C–O stretching vibrations, which may be associated with alcohols, esters, or ether linkages. In addition, the peaks in the region of 942.66–827.21 cm^{-1} suggest aromatic C–H bending, indicating the possible presence of substituted aromatic compounds. Overall, the FTIR profile suggests the presence of hydroxyl, aromatic, and oxygen-containing functional groups, which are commonly reported in bioactive secondary metabolites and may contribute to the observed antibacterial activity [47].

3.7.2. Gas chromatography–mass spectrometry

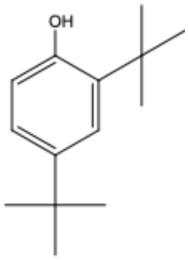
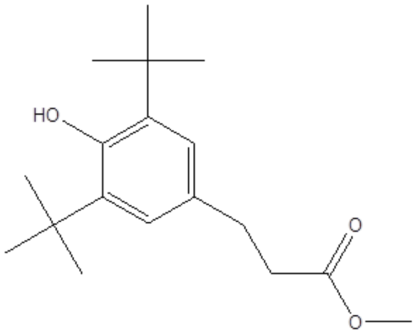
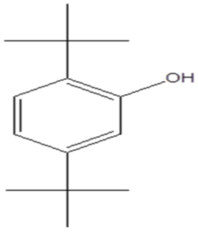
GC–MS analysis of the partially purified bioactive fraction revealed several putatively identified compounds by comparing their mass spectra with those available in the NIST Mass Spectral Library [46].

Among the detected constituents, phenolic compounds such as 2,4-di-tert-butylphenol and related compounds have previously been reported to possess antimicrobial activity [47,48]. These compounds were identified as constituents of the partially purified bioactive fraction recovered after fermentation. Although these compounds were detected at relatively low abundances, previous studies have reported their antibacterial properties. In the present study, they are reported as putatively identified constituents of the partially purified bioactive fraction. The observed antibacterial activity is therefore associated with the partially purified bioactive fraction, while the contribution of individual compounds requires further investigation. Among the three putatively identified compounds, only 2,4-di-tert-butylphenol accounted for 1.16% of the total peak area in the chromatogram (Fig. 10, Table 1). It has been widely reported that secondary metabolites, particularly phenolic compounds, can exhibit antibacterial activity even at low concentrations [46] due to their ability to disrupt microbial cell membranes and interfere with essential cellular processes. Although sweet potato peel contains naturally occurring phenolic constituents, GC–MS analysis in the present study was performed on the partially purified bioactive fraction obtained after fermentation with *S. diastaticus*. The identified compounds therefore represent constituents of the partially purified bioactive fraction associated with the observed antibacterial activity. *Streptomyces* species are well recognized for their ability to synthesize structurally diverse secondary metabolites and to biotransform plant-derived substrates into bioactive derivatives [49,50]. Accordingly, the detected compounds may represent microbial metabolites and/or biotransformation products generated during the fermentation process. However, in the absence of appropriate fermentation and analytical blank controls, the origin of individual compounds, particularly, 2,4-di-tert-butylphenol, cannot be assigned exclusively to microbial metabolism. The antibacterial activity exhibited by the partially purified bioactive fraction further supports the presence of fermentation-associated bioactive metabolites recovered from the culture.

3.7.3. Nuclear magnetic resonance

The ^{1}H-NMR spectrum of the partially purified bioactive fraction extracted from *S. diastaticus* (Fig. 11) revealed signals at δ 0.98–1.54 ppm, corresponding to aliphatic methyl and methylene protons. A signal at δ 2.6 ppm may be attributed to protons adjacent to carbonyl or aromatic groups, while the resonance at δ 3.71 ppm suggests the presence of methoxy or oxygenated functional groups (–O–CH_3 or –CH–O–). These spectral features indicate the presence of aliphatic and oxygenated structural moieties within the partially purified bioactive fraction, which are commonly encountered in microbial secondary metabolites [51]. However, the presence of specific structural features

Table 1. Putatively identified bioactive compounds detected in the partially purified bioactive fraction from *S. diastaticus* by GC–MS analysis.

S. no.	Putatively identified compound	Molecular weight	Molecular formula	Reported biological activity
1.	2,4-Di-tert-butylphenol 	206	$C_{14}H_{22}O$	Antibacterial activity
2.	Benzenepropanoic acid, 3, 5-bis (1,1-dimethylethyl)-4-hydroxy-methyl Ester 	292	$C_{18}H_{28}O_3$	Antibacterial activity
3.	Phenol, 2,5-bis (1,1-dimethylethyl). 	206	$C_{14}H_{22}O$	Antibacterial activity

or individual bioactive compounds cannot be conclusively established based solely on ^{1}H-NMR analysis and requires further purification together with comprehensive spectroscopic analyses, including two-dimensional NMR and mass spectrometry, for structural elucidation. The observed antibacterial activity is therefore associated with the partially purified bioactive fraction as a whole, while the contribution of individual compounds requires further investigation.

3.8. Antibacterial Activity of the Partially Purified Bioactive Fraction Extracted from *S. diastaticus*

Antibacterial activity of the partially purified bioactive fraction extracted from *S. diastaticus* was evaluated against three bacterial pathogens, *E. coli*, *K. pneumoniae*, and *S. flexneri*. Figure 12 shows the dose-dependent antibacterial activity of the partially purified bioactive fraction against the tested bacterial pathogens. A dose-dependent increase in the inhibition zone was observed as the dose of the partially purified bioactive fraction increased from 20 to 60 µg per well (Table 2). The highest antibacterial activity of the partially purified bioactive fraction was observed at 60 µg, producing inhibition zones of 17 mm against *K. pneumoniae*, 16 mm against *E. coli*, and 14 mm against *S. flexneri*. Commercial antibiotic discs were included as positive controls for reference. No inhibition zones were observed for the selected antibiotics against *K. pneumoniae* under the present experimental conditions; therefore, no inference regarding bacterial susceptibility or resistance was made. The inhibition zones observed in this study were slightly higher than those reported in previous studies involving *Streptomyces* species, which showed lower inhibition zones [52]. These findings demonstrate the antibacterial potential of the partially purified bioactive fraction against the tested pathogenic bacteria.

Recent studies have reported antibacterial activities from diverse antimicrobial agents, including metal complexes [53] and biosynthesized ZnO nanoparticles [54]. These studies highlight the continued interest in developing novel antimicrobial substances to combat pathogenic microorganisms and AMR. Although all the test pathogens used in the present study are Gram-negative bacteria,

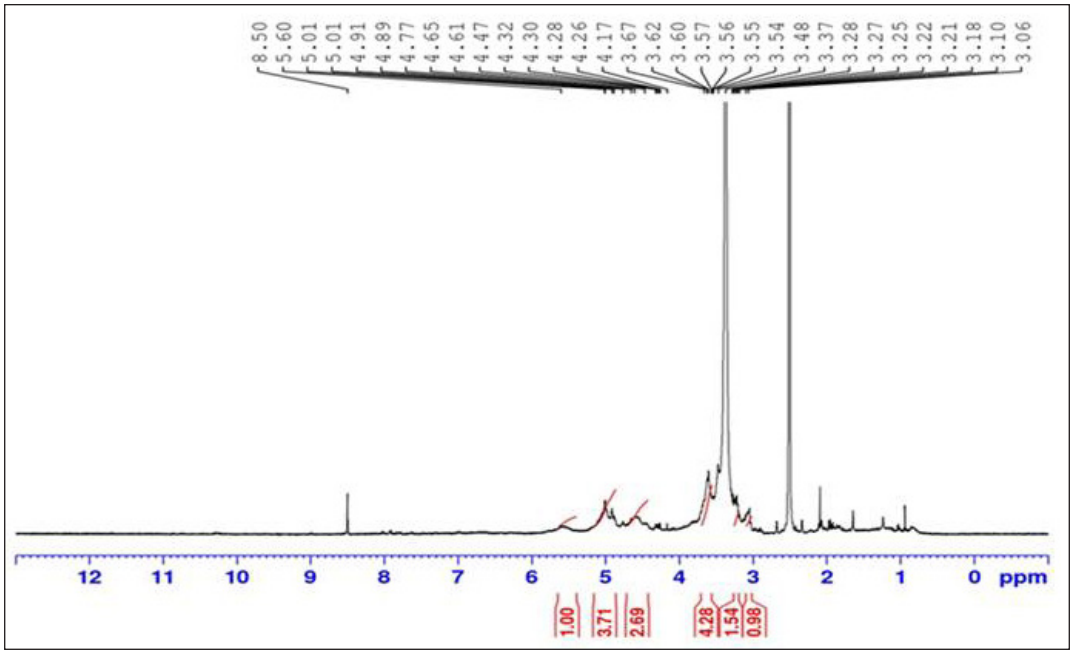


Figure 11. ^{1}H-NMR of partially purified bioactive fraction extracted from *S. diastaticus.*

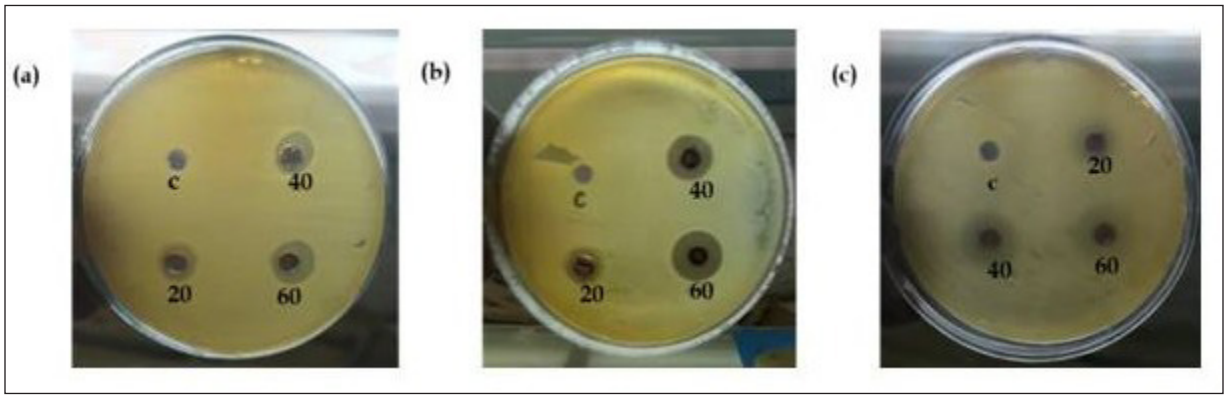


Figure 12. Antibacterial activity of partially purified bioactive fraction obtained from *S. diastaticus* against human pathogens (a) *S. flexneri* (b) *E. coli* and (c) *K. pneumoniae.*

Table 2. Zone of inhibition against human pathogens.

Zone of inhibition (mm)							
Test pathogens	Partially purified bioactive fraction			GEN	TET	AMP	KAN
	20 µg	40 µg	60 µg				
E. coli	11	14	16	20	9	20	19
K. pneumoniae	14	16	17	-	-	-	-
S. flexneri	11	12	14	12	9	9	12

GEN - Gentamicin, TET - Tetracycline, AMP - Ampicillin, KAN - Kanamycin. (-) - No zone of inhibition observed.

differences in susceptibility may be associated with variations in their cell envelope characteristics, including differences in outer membrane permeability and intrinsic resistance mechanisms such as efflux pumps.

The observed antibacterial activity against *E. coli*, *K. pneumoniae*, and *S. flexneri* demonstrates the potential of the bioactive metabolites produced by *S. diastaticus* as promising antimicrobial agents. The antibacterial activity may be associated with the putatively identified bioactive agents detected by GC–MS analysis, including phenolic and related compounds previously reported to exhibit antimicrobial properties. These compounds may contribute to antibacterial activity through interactions with bacterial cell membranes and intracellular components, potentially disrupting membrane integrity and essential cellular processes, ultimately leading to bacterial cell death [55]. Furthermore, these findings highlight the value of sweet potato peel waste as a sustainable substrate for the production of bioactive metabolites and support the potential application of agro-waste-based fermentation for the development of value-added antimicrobial products. The results provide a scientific basis for future investigations aimed at the discovery and development of novel antimicrobial agents from sustainable microbial resources.

4. CONCLUSION

This study demonstrated the feasibility of utilizing sweet potato peel waste as a sustainable substrate for the production of bioactive metabolites by *S. diastaticus*. Among the agro-wastes evaluated, sweet potato peel waste supported the highest growth and crude metabolite yield. The partially purified bioactive fraction exhibited antibacterial activity against *E. coli*, *K. pneumoniae*, and *S. flexneri*, with the highest inhibition observed against *K. pneumoniae*. FTIR, GC–MS, and ^{1}H-NMR analyses indicated the presence of putatively identified bioactive metabolites associated with the observed antibacterial activity. The novelty of the present study lies in demonstrating the suitability of sweet potato peel waste as a fermentation substrate for the production of bioactive metabolites by *S. diastaticus* and in characterizing the resulting partially purified bioactive fraction associated with antibacterial activity against human pathogens. These findings highlight the potential of agro-waste valorization as an environmentally sustainable approach for the production of value-added microbial metabolites with antibacterial properties.

The present study focused on the production, partial purification, characterization, and antibacterial evaluation of metabolites produced by *S. diastaticus* using sweet potato peel waste as a sustainable substrate. Although the bioactive metabolites were characterized using FTIR, GC–MS, and ^{1}H-NMR analyses, further studies are required for the complete purification and structural elucidation of the bioactive metabolites. In addition, the antibacterial activity was evaluated against selected bacterial pathogens, and broader investigations may provide further insights into their antimicrobial potential. Furthermore, optimization and scale-up of the fermentation process using agro-waste substrates may facilitate the development of cost-effective and environmentally sustainable approaches for bioactive metabolite production. The findings of this study provide a foundation for future research on agro-waste valorization and microbial bioprocesses for the production of valuable antimicrobial metabolites.

5. ACKNOWLEDGMENT

The authors acknowledge the Environmental Microbial Biotechnology Laboratory, Department of Environmental Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India, for providing the research facilities and continuous support throughout this study.

6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FUNDING

Peacebenedicta W. Komba acknowledges the Open University of Tanzania and the Higher Education for Economic Transformation (HEET) Project under the Ministry of Education, Science and Technology, Tanzania, for supporting her Ph.D. studies.

8. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVAL

This study did not involve experiments on human participants or animals. Therefore, ethical approval was not required.

10. DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

11. DISCLOSURE OF THE USE OF AI TOOLS

The authors used ChatGPT (OpenAI) for language editing and formatting during manuscript preparation. The AI tool was not used for the generation, analysis, or interpretation of the research data or

for drawing scientific conclusions. All AI-assisted text was critically reviewed, verified for accuracy, and edited by the authors before inclusion in the manuscript. The authors take full responsibility for the final version of the manuscript.

12. PUBLISHER'S NOTE

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How to cite this article:
Komba PW, Priya BS. Agro-waste valorization of sweet potato peel waste for production and characterization of antibacterial bioactive metabolites from *Streptomyces diastaticus*. J Appl Biol Biotech 2026;14(6):96-106. DOI: 10.7324/jabb.2026.314800