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# Sustainable fungal production of pectinase in orange peel-based medium: Taguchi optimization, juice clarification and green synthesis of selenium nanoparticles for biomedical applications

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ABSTRACT

Pectinase is a commercially important enzyme that is extensively utilized in the food, textile, and paper industries; yet, its large-scale production poses a hurdle due to high cost of pectin for its production. This research utilized Taguchi optimization to improve pectinase production from a non-aflatoxigenic local strain of *Aspergillus flavus*, using orange peel as an economical substrate. The improved pectinase was studied for juice clarification and eco-friendly synthesis of selenium nanoparticles (AFP-SeNPs), showcasing its enhanced biotechnological capabilities. The Taguchi L9 orthogonal optimization of pH, inoculum size, substrate concentration, and incubation time yielded a 397.7 % enhancement in pectinase production with maximum enzyme activity of 921.3 U/ml, which clarified orange juice by 76.6 %. Herein, we report the first study to synthesize SeNPs using pectinase which were spherical, crystalline, having sizes of 50.97–98.43 nm and absorbed maximally at 268 nm. The nanoparticles inhibited growth of multidrug-resistant bacterial pathogens (*Klebsiella oxytoca, Enterobacter cloacae*, and *Salmonella enterica*) up to 25.3 mm and total suppression of fungal growth (*Aspergillus flavus*, *Aspergillus niger*, and *Penicillium* sp.), while displaying 35.9–59.3 % DPPH radical scavenging activities. Additionally, AFP-SeNPs successfully prevented blood clot formation *in vitro*. This research which uniquely combines pectinase-assisted green production of SeNPs has broadened the applications of pectinase. The multifunctional bioactivities of AFP-SeNPs—antimicrobial, antioxidant, and anticoagulant—underscore their significance in biomedicine, pharmaceuticals, and industrial biotechnology. These findings would enhance sustainable enzyme manufacturing and environmentally friendly nanotechnology, providing a cost-efficient and scalable approach for future developments.

## 1. Introduction

Pectinase, a category of enzymes that facilitate the breakdown of pectin, is essential in numerous industrial applications, especially in the food, textile, and paper sectors. Pectin, a structural polysaccharide primarily consisting of galacturonic acid units, is essential to plant cell walls to enhance the rigidity and stability of plant tissues and also cellular communication (Shin et al., 2021). The enzymatic degradation of pectin by pectinases promotes industrial processes including fruit juice clarity, wine production, textile retting, and paper pulping, hence improving product quality and yield (Arshad et al., 2020; Lhamo et al., 2022; Rashima et al., 2022). The economic significance of pectinase has prompted substantial research focused on enhancing its production and application, especially through the use of fungal species recognized for their superior pectinase production ability.

Fungi, particularly from the genera *Aspergillus* and *Penicillium*, are

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noted for their proficient secretion of extracellular enzymes, including diverse pectinases such as polygalacturonase, pectin lyase, and pectin esterase (Haile and Ayele, 2022). These enzymes specifically cleave bonds within the pectin molecule, resulting in its breakdown into simpler molecules (Dasari, 2020; Vaz et al., 2020; Wagh et al., 2022). The capacity of these fungi to generate a variety of pectinases renders them especially beneficial for industrial applications, where distinct pectinase types may be necessary to get optimal outcomes in diverse processes (Ahmed and Rahman, 2021; Jalil et al., 2023). As with several bioprocesses, efficient production of pectinase by microorganisms entails the exploitation of suitable growth medium and strategies to optimize the process parameters for yield improvement. These include the increasing use of nutrient-rich agro-industrial wastes for the cost-effective production of microbial products in the bourgeoning field of circular bioeconomy (Elegbede et al., 2021; Ajayi and Lateef, 2023; Lateef, 2023; Yusuf-Salihu et al., 2024).

Optimizing pectinase production is essential for cost reduction and efficiency enhancement in industrial applications, as it directly influences total productivity and economic viability in processes dependent on pectin breakdown. Conventional techniques for enhancing enzyme production typically modify one variable at a time, which is a laborious and time-consuming strategy that overlooks the interactions among numerous parameters (Moharram et al., 2022). Conversely, contemporary statistical techniques, such as the Taguchi design of experiment (DOE), facilitate the concurrent assessment of many variables, yielding a more thorough comprehension of the factors influencing pectinase production (Qi et al., 2020; OĞUZ and Akarslan, 2022). The Taguchi method utilizes an orthogonal array to systematically alter experimental parameters, rendering it especially beneficial in biotechnological processes that need consideration of complicated interactions. This approach not only discerns the primary effects of many components but also mitigates the impact of noise, resulting in robust and dependable outcomes (Nouri and Khodaiyan, 2020). The Taguchi technique has been effectively utilized to optimize the production of numerous enzymes, such as tannase, alkaline protease, xylanase, and α-amylase, underscoring its versatility and efficacy (Elegbede and Lateef, 2019; Qi et al., 2020; OĞUZ and Akarslan, 2022; Ajayi and Lateef, 2025a, b). The application of this process to pectinase synthesis could be essential for optimizing enzyme yield and reducing production costs for sustainable enzyme manufacturing.

The global pectinase market has witnessed substantial expansion, propelled by rising demand in the food and beverage sector, where pectinase is employed for juice extraction and clarification operations. In 2022, the market was valued at roughly $18.3 billion and is anticipated to reach around $26.6 billion by 2030, reflecting a compound annual growth rate (CAGR) of approximately 11.1 % throughout the projection period (Gasani et al., 2021; Moharram et al., 2022; Zion Market Research, 2023). This expansion highlights the necessity of effective manufacturing methods to provide this essential enzyme for many applications.

In the industrial production of pectinase, pectin is required as the substrate to spur the microbial strain for the induced production of pectinase. However, due to the high cost of pectin, agro-wastes are now being investigated as cheaper sources of substrates in the microbial production of pectinase (Shrestha et al., 2021). Among such agro-wastes that have been considered as cheap sources of pectin is the orange peel. *Citrus* is a group of fruit-bearing plants in the family Rutaceae of which members include lemon, orange, grape, tangerine and lime. They are widely cultivated for their fruits which produce juices that taste from sour to being sweet. The juice contains vitamins, minerals, crude fibre, flavonoids and several bioactive compounds that are important for good health and ethnomedicinal values (Lu et al., 2023). In processing fruits of *Citrus,* huge wastes are produced in the forms of peel, fibre, pomace and seeds. Of these, the peel accounts for about 30–34 % (Rafiq et al., 2018). Being rich in nutrients, the *Citrus* peels have been valorized into different products that include microbial production of enzymes, biofuel, and as sources of antioxidants, pectin and essential oils (Suri et al., 2022). Among *Citrus* plant, sweet orange (*Citrus sinensis*) accounts for about 82 % as the most cultivated with major producing countries being Brazil, China, EU, Mexico, Egypt, and US (Villa, 2025). In Africa, the major producers of oranges are Egypt, South Africa, and Morocco. Nigeria produced 4229,445.99 tons of citrus fruits in 2023 out of a total of about 17.5 million tons produced in Africa as reported by FAOSTAT (2025). Thus, the abundant peel from processing of orange which is rich in pectin can be valorized by microorganisms to produce pectinase. Some studies have reported pectin yield of 12.52–22.45 % from sweet orange peel (Kamal et al., 2021).

In nanobiotechnology, there are increasing efforts to expand the capabilities of bioresources towards sustainable production of nanomaterials via eco-friendly approaches. Therefore, enzymes have taken centre stage to produce different nanoparticles within size range of 1–100 nm for diverse applications (Adelere and Lateef, 2016; Elegbede and Lateef, 2021). In the past, we have explored keratinase, laccase, and xylanase to produce Ag, Au, ZnO, CaO, and Ag-Au nanoparticles (Lateef and Adeeyo, 2015; Lateef et al., 2015; Elegbede et al., 2018; Elegbede et al., 2019; Elegbede et al., 2020; Elegbede et al., 2024; Lateef et al., 2025). However, there is dearth of information on green synthesis of nanoparticles using pectinase as only a study reported the synthesis of Au nanoparticles by the enzyme (Zhang et al., 2017). Therefore, there is need to extend the values of pectinase in nanobiotechnology to synthesize nanoparticles. Among the emerging important nanoparticles of extensive biomedical values is selenium nanoparticles (SeNPs) with proven antimicrobial, antioxidant, anticancer, anti-diabetic and anti-inflammatory activities (Bisht et al., 2022).

From the foregoing, the present study was designed for bioprospecting new high-yielding fungal strains for the sustainable production of pectinase, and utilization for fruit juice clarification, and eco-friendly synthesis of selenium nanoparticles for some biomedical applications. The study evaluated the suitability of orange peel for the fungal production of pectinase, and its optimization using the Taguchi technique. Thereafter, investigation of pectinase for the synthesis of selenium nanoparticles was carried out for the first time, while the SeNPs were further studied for their biomedical applications; antimicrobial, antioxidant, and anticoagulant activities. The novel approach underscores the capabilities of pectinase in biotechnological applications and paves the way for further research and development in nanotechnology that could enhance prospective uses in pharmaceuticals, biotechnology, and nanomedicine. The scope of the study has relevance to some of the targets of the UN sustainable development goals, particularly good health and well-being (SDG 3), industry, innovation and infrastructure (SDG 9), and responsible production and consumption (SDG 12).

## 2. Materials and methods

### 2.1. Substrate

Orange fruits were bought from Oja Oba Market in Ilorin, Kwara State (8.4966 N, 4.5425 E). It was cleaned, peeled, and the peels were dried in a hot air oven at 60 °C until a constant weight was achieved. Following drying, the peels were milled and sieved (0.5 mm) to produce a fine powder, which was subsequently stored in an airtight container until needed. Pectin was extracted from orange peel following the methods of Pokhrel and Dahal (2024). Precisely 5 g of dried peel powder was mixed with 150 ml of distilled water and pH of the mixture was modified to 2.0 with dilute hydrochloric acid. The acidic mixture was subsequently heated to 80 °C and sustained at this temperature for 60 min, and thereafter allowed to cool to room temperature. The liquid extract was subsequently separated from the solid residue using Whatman No. 1 filter paper. The filtrate, containing solubilized pectin, was precipitated with 95 % ethanol. The mixture was permitted to remain undisturbed for 2 h at ambient temperature to ensure the complete

precipitation of pectin. The precipitated pectin manifested as a gelatinous substance was skimmed off. Filtration was subsequently performed to separate the coagulated pectin. The coagulated pectin was dried in a hot air oven at 50 °C for 24 h. The percentage yield of pectin was computed as follows:

$$\%\text{yield of pectin} = \frac{\text{Weight of pectin extracted}}{\text{Weight of peel powder taken}} \times 100\% \quad \text{Eqn. 1}$$

### 2.2. Isolation and identification of fungal isolate

The fungal strain used for this work was isolated from deteriorated lime peels using direct inoculation method. The microscopic structures of the mold were observed by staining with lactophenol cotton blue and assessed for the microscopic characteristics (Rudhra et al., 2024). The molecular identification was based on the use of ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATT GATATGC-3′) primers for the amplification of the genomic DNA following established techniques (Cheng et al., 2021). The PCR products were analyzed on agarose gel electrophoresis and subsequently sequenced at a commercial facility. Sequence alignment was performed using BioEdit software, and the consensus sequences were subsequently submitted to GenBank of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for isolate identification and obtainment of accession number. Furthermore, MEGA-11 software was used to construct the phylogenetic tree of the isolate (Tamura et al., 2021).

### 2.3. Inoculum development

A loopful of fungal mycelium was inoculated into sterile 50 ml inoculum medium (comprising 0.2 % yeast extract, 1 % sucrose, pH 5.5). The inoculum was cultivated at 30 ± 2 °C on a shaker at 100 rpm for 24 h (Elegbede et al., 2024).

### 2.4. Qualitative pectinase assay of the fungal isolate

Pectinase producing ability of fungal isolate was determined using pectinase screening agar medium (PSAM). The PSAM contains the following (in g/l): $(NH_4)_2HPO_4$, 3.0; $KH_2PO_4$, 2.0; $K_2HPO_4$, 3.0; $MgSO_4$, 0.1; pectin, 10.0; and agar, 20.0. A hole was bored at the center of the plate using a 6 mm-diameter corkborer. An actively growing fungal isolate disc was inoculated into the pre-welled plate and incubated for 48 h. Following incubation, the plate was flooded with an iodine-potassium iodide solution (5.0 g potassium iodide and 1.0 g iodine in 330 ml of distilled water) (Adedayo et al., 2021). The plate was examined for the appearance of a clear zone, which served as an indicator of qualitative pectinolytic activity, and the diameter of the zone was measured using a meter rule.

### 2.5. Pectinase production in submerged fermentation

The basal medium formulation for pectinase production via submerged fermentation included the following components (g/l): Orange peel 10.0; $(NH_4)_2HPO_4$, 3.0; $KH_2PO_4$, 2.0; $K_2HPO_4$, 3.0 and $MgSO_4$,0.1. The orange peel served as a complex substrate that supplied pectin. In a 250 ml flask containing 40 ml of basal medium, the sterile medium was inoculated with 10 % (v/v) of a 24h-old fungal inoculum. Subsequently, incubation occurred at 30 ± 2°C for 120 h on a shaker at 100 rpm. At 24-h interval, the pH was assessed, and the contents were filtered using Whatman No. 1 filter paper, followed by centrifugation of the filtrate at 4000 rpm for 25 min. The cell-free supernatant served as the crude enzyme and was stored at 4 °C until needed.

### 2.6. Quantitative pectinase assay

About 1 ml of 1 % pectin prepared in 0.1 M sodium acetate buffer at pH 5.5 and of 1 ml crude enzyme was mixed in a sterile tube and incubated in a water bath at 50 °C for 30 min. After incubation, 1 ml of the DNS (dinitrosalicylic acid) reagent was added to stop the hydrolysis reaction. The mixture was shaken to mix the content and then placed in a boiling water bath for 10 min. About 5 ml of distilled water was then added and allowed to cool. The absorbance was read at 540 nm using a spectrophotometer. The blank consisted of 1 ml of 0.5 % pectin, 1 ml of sodium acetate buffer (0.1 M, pH 5.5) and 1 ml of DNS reagent. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 µmol of galacturonic acid per minute under standard assay conditions (Sudeep et al., 2020).

$$\text{Enzyme activity}(\text{U}/\text{ml}) = \frac{\mu\text{g of galacturonic acid}}{194.1 x v x t} \quad \text{Eqn. 2}$$

where “v” is the enzyme volume used in the assay, “194.1” is the molecular weight of galacturonic acid, and t is the reaction time in min (Patidar et al., 2018).

### 2.7. Optimization of the production of pectinase using the Taguchi technique

Taguchi method was employed in investigating the relationships that exist among the variables of medium components and to optimize for higher yields of enzyme production. Taguchi design methodology was performed using modified steps as stated in Fig. 1, and it was used to set up the critical fermentation factors: initial pH, inoculum size (%), substrate (orange peel) concentration (g/l) and fermentation time (h). The effective levels were set as low, intermediate and high (Table 1). Conditions such as temperature (30 ± 2 °C), agitation (100 rpm), and media: flask volume ratio (40 ml/250 ml flask) were not changed and fixed as standard conditions for the fermentation.

The appropriate Taguchi design arrangement for data analysis was selected. The L9-orthogonal array method based on modified methods described by Taguchi (1986), which overcomes many problems related with conventional methodology, was used for the aforementioned control parameters with three levels of factor variation. In Taguchi method, the summary statistic $\eta$ is called signal to-noise ratio (SNR). The value of $\eta$ is an effective indicator for the assessment of the impact of process parameters on enzyme synthesis. The larger-the-better signal-to noise was utilized to evaluate the summary statistic $\eta$ in the present study, because an improved production of pectinase was targeted, and it was defined as follows: (Chou et al., 2003)

$$\text{SNR } (\eta) = -10\log(R^{-2}). \quad \text{Eqn. 3}$$

Where, SNR represents the signal-to-noise ratio, while R represents the response or yield of correspondent trials. For each component, the optimal conditions give the largest SNR ratio.

The optimal combination of fermentation parameters was selected using the effect table, and the result was predicted using Eqn. 3. To validate or confirm the optimized methodology, fermentation experiments were run in duplicates and the samples collected were assayed for pectinase production. ANOVA was performed to evaluate the fermentation parameters that were statistically significant.

$$Ypr = Yave + \sum_{i=1}^{n=5} (Yopt - Yave) \quad \text{Eqn. 4}$$

Where, $Y_{pr}$ is the predicted pectinate activity; $Y_{ave}$ is the mean of output values; and $Y_{opt}$ is the optimum output value

### 2.8. Determination of aflatoxigenic potential of fungal isolate

The fungal isolate was seeded into neutral red desiccated coconut agar (NRDCA) as per the protocols established by Atanda et al. (2011) and cultured at ambient temperature for 48 h for rapid detection of

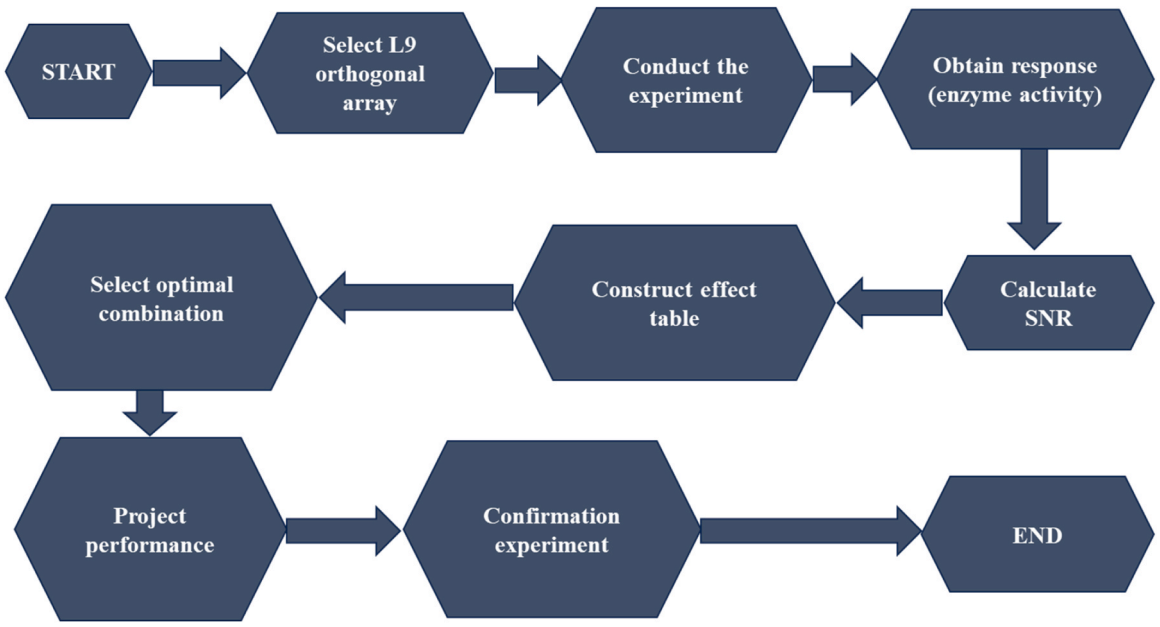



**Fig. 1.** Work flow for Taguchi bioprocess optimization for pectinase production.

**Table 1**
Variation levels of the parameters for the optimization of pectinase.

| Parameters | Designation | Levels of variation | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Initial pH | A | 4.5 | 5.5 | 6.5 |
| Inoculum size (%) | B | 5 | 12.5 | 17.5 |
| Orange peel conc. (%) | C | 1 | 1.5 | 2.5 |
| Fermentation time (h) | D | 24 | 48 | 72 |

aflatoxigenic fungi, through the production of yellow pigmentation on the red agar. The fungal growth on NRDCA plates was subsequently examined under UV light (365 nm) for fluorescence intensity to detect the presence of aflatoxigenic fungal strains (Atanda et al., 2011).

### 2.9. Biotechnological applications of pectinase

#### 2.9.1. Clarification of fruit juice

Fresh orange (*Citrus sinensis*) juice was subjected to treatment with crude enzyme at a ratio of 9:1 ml (juice-to-pectinase) following the methods of Elegbede and Lateef (2018) in a test tube, whereas the control sample received distilled water in place of crude pectinase. The sample was incubated at ambient temperature for 2 h, and the degree of clarity was assessed by measuring the height of the clear juice. The percentage was calculated thus:

$$\%\text{Juice clarification} = \frac{\textit{Height of clear juice}}{\textit{Total height of juice}} \times 100 \qquad \text{Eqn. 5}$$

### 2.10. Biosynthesis and characterization of SeNPs using pectinase

Crude fungal pectinase was employed to biosynthesize selenium nanoparticles (SeNPs). Precisely, 5 ml of the enzyme was combined with 30 ml of 10 mM sodium selenite. The reaction mixture was incubated at ambient temperature for 48 h, and the synthesis of SeNPs was assessed by observing the color change of the solution.

#### 2.10.1. Characterization of pectinase-mediated SeNPs (AFP-SeNPs)

The AFP-SeNPs were characterized by analyzing the absorbance spectra using a UV-Vis spectrophotometer within the range of 200–800 nm. Furthermore, to ascertain the biomolecules implicated in the biosynthesis, capping, and stability of the nanoparticle, Fourier transform infrared (FTIR) spectroscopy was conducted. The morphology of the biosynthesized SeNPs were assessed via transmission electron microscopy (TEM), while the crystallinity of the nanoparticles was evaluated using selected area electron diffraction (SAED).

#### 2.10.2. Antimicrobial activities of AFP-SeNPs

The agar well diffusion method was utilized to assess the antibacterial effectiveness of AFP-SeNPs against multidrug-resistant strains of *Klebsiella oxytoca*, *Enterobacter cloacae*, and *Salmonella enterica* sourced from the University of Ilorin Teaching Hospital, Ilorin, Nigeria. Bacterial suspensions, calibrated to a 0.5 McFarland standard ($1 \times 10^6$ cfu/ml), were evenly distributed on Mueller-Hinton agar plates. Sterile cork borer was utilized to perforate wells on agar plates, which were then filled with 100 µl of 50–200 µg/ml of the biosynthesized AFP-SeNPs. Distilled water was used in place of nanoparticles as the negative control. The plates were incubated at 37°C for 18–24 h, and the inhibition zones around each well were measured in millimeters.

The antifungal effectiveness of the nanoparticles against specific economically significant fungi was evaluated using the methods developed by Yusuf-Salihu et al. (2025). Concentrations of AFP-SeNPs (50 and 100 mg/ml) were incorporated into potato dextrose agar (PDA) plates, onto which 6 mm agar plugs from 48h-old cultures of *Aspergillus flavus*, *Penicillium* sp., and *Aspergillus niger* were placed. PDA plates inoculated with fungal agar plugs lacking nanoparticles functioned as the control group. All plates were incubated at 28 ± 2 °C for a duration of 48 h. The diameters (D) of the fungal growth were quantified in millimeters, and the percentage of growth inhibition was computed correspondingly.

$$\%\text{Fungal growth inhibition} = \frac{\textit{DControl} - \textit{DTest}}{\textit{DControl}} \times 100 \qquad \text{Eqn. 6}$$

#### 2.10.3. Anticoagulant activities of AFP-SeNPs

The anticoagulant activity of AFP-SeNPs was assessed using the method described by Yusuf-Salihu et al. (2025). In this procedure, 1 ml of 100 µg/ml nanoparticles was introduced into 0.5 ml of freshly drawn blood from a healthy donor following ethical guideline. In the positive control, 0.5 ml of fresh blood in an EDTA tube was utilized, whereas in the negative control, 0.5 ml of blood in a sterile Eppendorf tube was

employed. After incubation at room temperature for 3 h, the tubes were visualized for blood coagulation and captured with a digital camera and then observed under the microscope (×40).

#### 2.10.4. DPPH radical scavenging assay of AFP-SeNPs

The capacity of AFP-SeNPs to scavenge free radicals was evaluated by combining 4 ml of 0.3 mM methanolic DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich) with 1 ml of graded doses of AFP-SeNPs at 5, 10, 20, 40, and 60 µg/ml, followed by incubation in the dark for 30 min at room temperature (Yusuf-Salihu et al. (2025). The optical densities were measured using a Spectro UV-2505 spectrophotometer at 517 nm, and the percentage scavenging activity was calculated accordingly:

$$\%DPPH\ scavenging\ effect = \frac{AD - AS}{AD} \times 100 \quad \text{Eqn. 7}$$

Where AD is the blank's absorbance, AS is the sample's absorbance.

## 3. Results and discussion

### 3.1. Identification of the fungal isolate

This work identified the fungal strain of *Aspergillus flavus* KW03 as a pectinase producer by a combination of morphological and molecular approaches. Preliminary macroscopic and microscopic examinations indicated its categorization within the genus *Aspergillus*, characterized by greenish colony pigmentation, radial growth, septate hyphae, biseriate conidial heads, and roughened conidia, aligning with traditional descriptions of *A. flavus* (Fig. 2a). The Internal Transcribed Spacer (ITS) region, a well-established molecular identifier for fungal species, was sequenced to validate this identification. Sequence alignment demonstrated complete identity to *A. flavus*, and phylogenetic analysis placed the isolate inside a robust clade of *A. flavus* strains, indicating a close genetic affinity to other species members (Fig. 2b). The strain was designated as *A. flavus* KW03, and its ITS sequence submitted to GenBank with the accession number OR461563. The integration of morphological and molecular methodologies substantiated the taxonomic classification of the isolate and affirmed its positioning within the *A. flavus* clade.

### 3.2. Extraction of pectin from orange peel

The orange peel yielded 16.4 % pectin using the acid hydrolysis method. Studies have reported yield of 3.82–46.09 % using different solvents and techniques for extraction of pectin from orange peel (Duwee et al., 2022; Zioga et al., 2022; Turan et al., 2024), pointing to orange peel as a good renewable and sustainable source of pectin.

### 3.3. Qualitative assay of production of pectinase by A. flavus

A distinct zone of pectin hydrolysis was observed on the assay plate, measuring 38 mm surrounding the fungal colony, which indicated pectinolytic activity (Fig. 2c). The manifestation of the hydrolyzed region following iodine-potassium iodide treatment validated that the pectinase synthesized extracellularly by the fungus had degraded the pectin polymer backbone, yielding galacturonic acid and pectic oligosaccharides, depending upon the degree of polymerization. The observed hydrolysis zone exceeded the 15–32 mm range documented by Sudeep et al. (2020) for *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. Result obtained in the present study showed that orange peel could serve as an excellent sustainable substrate to produce pectin for the fungal synthesis of pectinase.

### 3.4. Unoptimized pectinase production

The isolate effectively utilized orange peel to produce pectinase, with enzyme activity reaching a maximum of 185.1 U/ml at 24 h, before declining to 100.93 U/ml at 48 h. Simultaneously, a decrease in pH was observed throughout the fermentation period. At 24 h, the pH was 4.86, but by 48 h, it dropped to 4.73. The reduction in pH indicates increased acidogenesis, likely resulting from the degradation of pectin into galacturonic acid and other acidic intermediates. The reduction of pH may

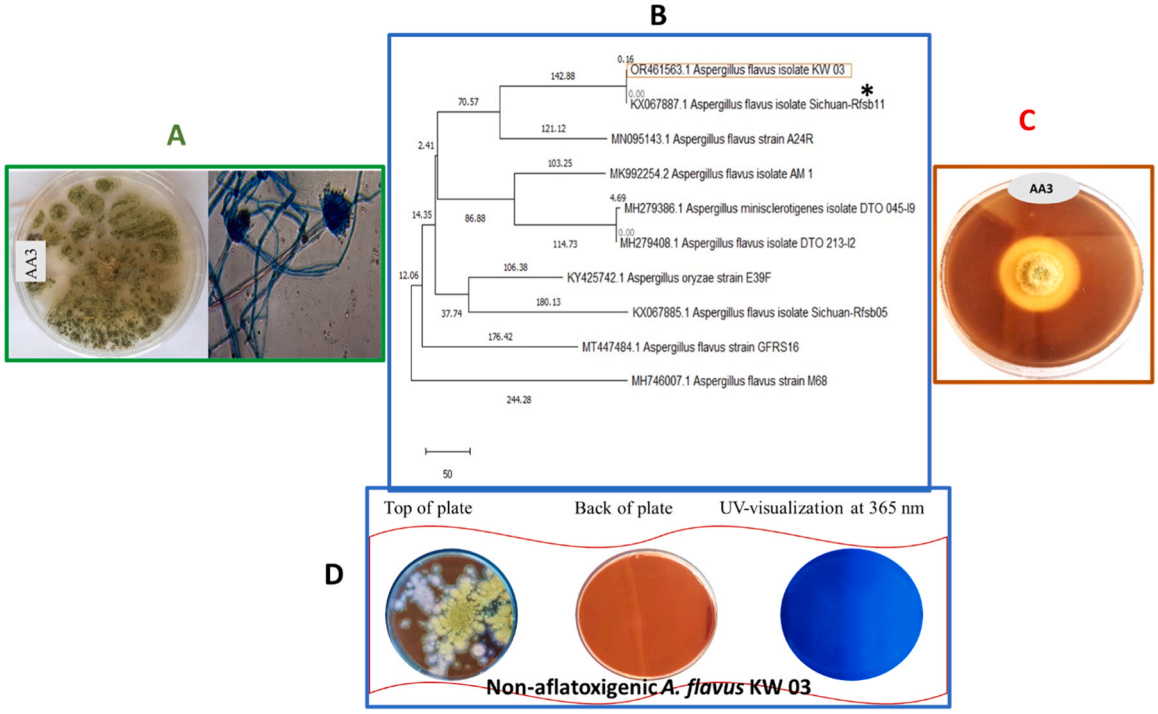



**Fig. 2.** The macro and micro-morphological features (A), phylogenetic tree showing genetic relatedness of *A. flavus* KW03 (B), pectinase qualitative assay of the strain (C), and non-fluorescence of the isolate on NRDCA for the detection of aflatoxin.

have led to the decrease in enzyme activity. The pH range corresponds with the ideal range of 4.0–6.5 for fungal pectinase synthesis as reported by Sheladiya et al. (2022).

Thus, pectinase was synthesized through submerged fermentation by *A. flavus* KW03 utilizing a minimal salt medium with pectin as the main carbon source. The selection of carbon source is pivotal in microbial metabolism. In large-scale fermentation, the economics of the process is greatly affected by the selection of a suitable and economical carbon source. The elevated expense of pure pectin renders its application for large-scale pectinase synthesis commercially unfeasible. Consequently, agricultural leftovers abundant in pectin, such as orange peels, are frequently suggested as alternative substrates.

### 3.5. Optimization of pectinase production through Taguchi method

In this study, the Taguchi L9 orthogonal array enhanced pectinase production by *Aspergillus flavus* KW03, demonstrating notable differences in enzyme synthesis across several fermentation conditions (Table 2). The maximum pectinase activity observed was 797.4 U/ml, indicating the local optimum conditions for enzyme synthesis by *A. flavus* KW03. This outcome demonstrates a significant enhancement in pectinase yield with 4.31-fold relative to the unoptimized condition. The synthesis of microbial metabolites is affected by diverse media, physiological, and biochemical variables, which vary across different microorganisms (Elegbede et al., 2024).

The data indicated that pectinase production is significantly influenced by fermentation factors and their corresponding levels. The Taguchi Design of Experiments (DOE) methodology offered a prediction strategy for optimizing pectinase yield utilizing effect tables. The signal-to-noise (S/N) ratio was employed to assess the impact of each experimental variable on enzyme production, with the effect table (Table 3) enabling the comparative evaluation of various parameter contributions. A change in signal results in a more significant effect on the output variable; thus, elevated S/N ratio values indicate a larger impact of the combined factors on enzyme production (Govindaraji and Vuppu, 2020).

The ideal fermentation profile for *A. flavus* KW03 was determined to be A3B3C2D1, which corresponds to an initial pH of 6.5, an inoculum size of 17.5 %, a substrate concentration of 1.5 %, and a fermentation time of 24 h (Table 4), and produced global optimum pectinase yield of 921.3 U/ml. The global maximum activity achieved from this improved combination demonstrated a 15.54 % enhancement compared to the local optimum of 797.4 U/ml obtained from the initial orthogonal array studies. The optimized pectinase production process yielded a total improvement of 397.7 % compared to the conventional unoptimized bioprocess. These results correspond with earlier research indicating that optimizing pectinase production through the Taguchi approach enhanced enzyme yields (Govindaraji and Vuppu, 2020). The noted enhancement in pectinase production is ascribed to the synergistic interactions among the evaluated fermentation parameters, thereby reinforcing the efficacy of the Taguchi method in microbial enzyme optimization (Govindaraji and Vuppu, 2020; Ajayi and Lateef, 2025a; Lateef et al., 2025).

**Table 3**
Effect table of pectinase production by *A. flavus* KW03.

| | Parameters | | | |
|---|---|---|---|---|
| Level of parameters | Initial pH | Inoculum size | Substrate concentration | Fermentation Time |
| 1 | 38.54823 | 40.85004 | 43.85234 | **48.11060** |
| 2 | 47.71578 | 46.69901 | 44.98333 | 42.82544 |
| 3 | **52.48864** | **51.20361** | **49.91699** | 47.81661 |

**Table 4**
Performance of selected optimal combination of parameters by *A. flavus* KW03 for pectinase production.

| Parameters | Levels |
|---|---|
| Initial pH | 6.5 |
| Inoculum size (%) | 17.5 |
| Substrate concentration (g/l) | 2.5 |
| Fermentation time (h) | 24 |
| Optimum profile | A3B3C3D1 |
| *Signal- to- noise ratio | 59.28802 |
| *Pectinase activities (U/ml) | 921.3 |
| Fold increase (local optimum Vs unoptimized) | 4.31 |
| Fold increase (global optimum Vs unoptimized) | 4.98 |

* Each value is an average of two readings. A: initial pH, B: Inoculum size, C: Substrate concentration, D: Fermentation time.

### 3.6. Analysis of variance (ANOVA)

The statistical analysis of pectinase production was performed using ANOVA to assess the importance of several fermentation parameters, including pH, inoculum size, substrate concentration (orange peel), and fermentation duration (Table 5). The results underscored the comparative impacts of various influences on enzyme production. Among the evaluated factors, pH was identified as the most significant component, accounting for 52.09 % of the total variation in pectinase activity. The F-value of 3.2613 and a *p-value* of 0.1099 indicate that pH exerted the most significant influence on enzyme production, ranking first among the parameters. This indicates that sustaining an appropriate pH level is essential for increasing pectinase production by the fungus. The inoculum size was the second most influential factor, accounting for 27.97 % of the difference in pectinase output. Despite its F-value (1.1647) and *p-value* (0.3738) suggesting a lower statistical significance compared to pH, its considerable contribution highlights its significance in enhancing the fungal growth and pectinase production.

The influence of orange peel content on pectinase synthesis was moderate, accounting for 10.79 % of the observed variation. The F-value (0.3629) and *p-value* (0.7099) suggest that substrate concentration has a lesser effect compared to pH and inoculum size. The fermentation time was determined to be the least impactful variable, contributing 9.15 % with an F-value of 0.3023 ($p = 0.7497$). This indicates that although fermentation duration impacts pectinase synthesis, its effect is negligible relative to other parameters.

The ANOVA results demonstrate that adjusting pH and inoculum size

**Table 2**
Taguchi optimization of pectinase production by *A. flavus* KW03 in orange peel-derived pectin based submerged fermentation.

| Experimental No. | Initial pH | Inoculum size (%) | Substrate concentration (%) | Fermentation time (h) | Pectinase activity (U/ml) | Signal- to- Noise |
|---|---|---|---|---|---|---|
| 1 | 4.5 | 5 | 1 | 24 | 42.7 | 32.60856 |
| 2 | 4.5 | 12.5 | 1.5 | 48 | 51.9 | 34.30335 |
| 3 | 4.5 | 17.5 | 2.5 | 72 | 273.3 | 48.73279 |
| 4 | 5.5 | 5 | 1.5 | 72 | 135.1 | 42.61311 |
| 5 | 5.5 | 12.5 | 2.5 | 24 | 483.6 | 53.68973 |
| 6 | 5.5 | 17.5 | 1 | 48 | 219.9 | 46.84450 |
| 7 | 6.5 | 5 | 2.5 | 48 | 232.5 | 47.32846 |
| 8 | 6.5 | 12.5 | 1 | 72 | 402.9 | 52.10395 |
| 9 | 6.5 | 17.5 | 1.5 | 24 | **797.4** | **58.03352** |

**Table 5**
Summary of ANOVA of main effects of fermentation factors on pectinase production.

| Factors | DF | SS | MS | F-value | *p*-value | Contribution (%) | Rank |
|---|---|---|---|---|---|---|---|
| pH | 2 | 301.1592 | 150.5796 | 3.261302 | 0.109994 | 52.08665 | 1 |
| Inoculum size (%) | 2 | 161.6982 | 80.84908 | 1.164719 | 0.373772 | 27.96632 | 2 |
| Orange peel concentration (%) | 2 | 62.40026 | 31.20013 | 0.362941 | 0.709915 | 10.79237 | 3 |
| Fermentation time (h) | 2 | 52.93126 | 26.46563 | 0.302316 | 0.749735 | 9.154667 | 4 |

would be crucial for maximizing pectinase output, whereas orange peel concentration and fermentation duration exert comparatively lesser effects. These findings underscore the significance of regulated fermentation conditions in improving enzyme production efficiency (Tarafdar et al., 2021).

### 3.7. Aflatoxigenic potential of the fungal isolate

The findings demonstrated that *A. flavus* KW03 did not generate any discernible pigment on NRDCA, and fluorescence was absent on both the front and reverse sides of the culture plates (Fig. 2d), hence affirming its non-aflatoxigenic character (Okayo et al., 2020). This discovery corresponds with earlier investigations which documented similar outcomes for several fungal isolates, including those strains of *A. flavus* identified as non-aflatoxigenic (Elegbede et al., 2018; Rangel-Muñoz et al., 2022). Thus, the non-aflatoxigenic nature of *A. flavus* KW03 could position the strain for biotechnological applications, such as enzyme production without safety concerns about the production of aflatoxins. The choice of NRDCA for detection of aflatoxin production is predicated on the apparent pigmentation of aflatoxigenic fungal colonies or development of fluorescence under UV light at a wavelength of 365 nm, which signifies aflatoxin formation (Mohammed, 2023). These characteristics can be investigated on an excellent medium, neutral red desiccated coconut agar (NRDCA) that was developed and validated for screening of aflatoxigenic fungi (Atanda et al., 2011).

Aflatoxins, a class of highly carcinogenic secondary metabolites generated chiefly by specific *Aspergillus* species, present considerable health hazards to humans and animals. These toxins possess mutagenic, hepatotoxic, teratogenic, and immunosuppressive characteristics, resulting in significant health risks when they contaminate food sources (Mateo et al., 2022; Shibabaw et al., 2023). Aflatoxin synthesis is not ubiquitous across all fungal strains; therefore, differentiating between aflatoxigenic and non-aflatoxigenic strains is essential for food safety and public health (Khan et al., 2021). This distinction is crucial for employing fungal strains in biotechnological applications, such as enzyme manufacturing like pectinase, where aflatoxin presence may jeopardize product safety (Rangel-Muñoz et al., 2022).

### 3.8. The effect of pectinase on clarification of orange juice

The pectinase derived from *Aspergillus flavus* KW03 clarified orange juice, resulting in a 76.6 % decrease in turbidity (Fig. 3). The efficacy of *A. flavus* KW03 pectinase in juice clarifying is due to its capacity to hydrolyze pectin, resulting in the liberation of soluble components and the sedimentation of insoluble particles. The enzymatic degradation of pectin promotes the clumping and settling of suspended particles, thus enhancing juice clarity and yield (Nouri and Khodaiyan, 2020; Lhamo et al., 2022) as obtained in the present study.

The clarifying of fruit juice is an essential phase in juice processing that improves visual appeal, stability, and consumer appeal by diminishing turbidity and haze that are caused by pectin. Pectinases are essential for the clearing of fruit juices, as they hydrolyze pectin, a structural polysaccharide at the α-1,4-glucosidic linkages to disintegrate cell wall in fruits. Recent studies on soursop, apple and barberry fruits have underscored the efficacy of diverse pectinase sources, including those from fungi and bacteria, in enhancing fruit juice extraction and quality by reducing turbidity and viscosity (Makebe et al., 2020; Nouri and Khodaiyan, 2020; Hosseini et al., 2021; Dowerah, 2023). The enzymatic activity not only liberates the juice but also lowers the water-retention capacity of pectin, facilitating enhanced extraction and clarifying of the juice (Makebe et al., 2020; Hosseini et al., 2021). The effective utilization of *A. flavus* KW03 pectinase in the clarification of orange juice underscores its potential for industrial applications, especially within the beverage sector, where there is a demand for high-quality, clear juices.

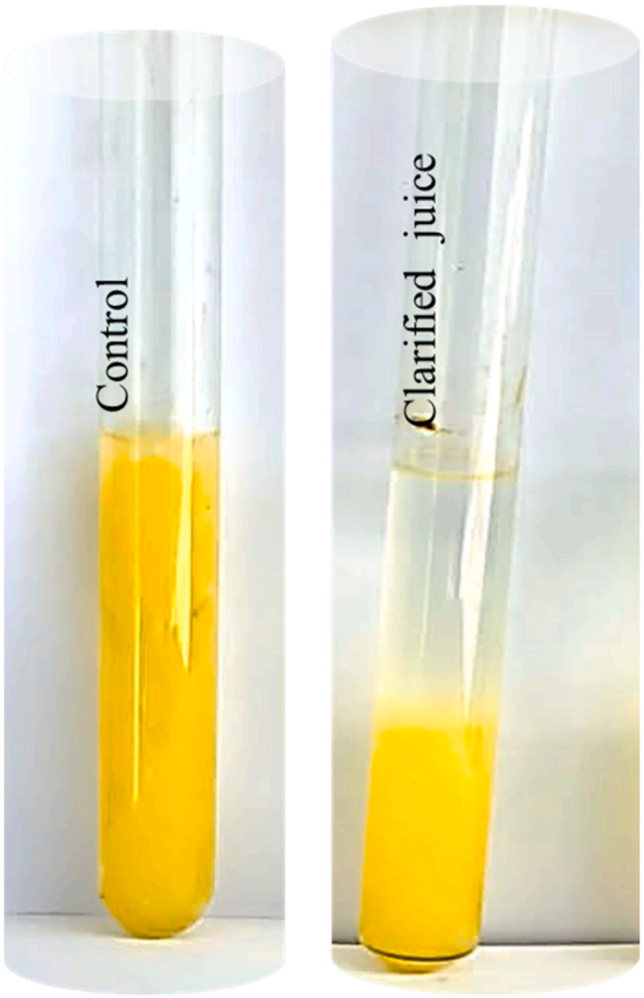



**Fig. 3.** The influence of the fungal pectinase on the clarification of orange juice.

### 3.9. Characteristics of AFP-SeNPs

The synthesis of SeNPs mediated by pectinase was verified by a discernible color change in the reaction mixture. Initially, the sodium selenite solution was colorless; but, after 72 h, it changed to a ruby red hue (Fig. 4a). The unique color change serves as a definitive indicator of selenium nanoparticle production, linked to the surface plasmon resonance (SPR) phenomena observed in nanoscale selenium. Comparable

findings have been documented in earlier investigations concerning the green synthesis of SeNPs, wherein the alteration of the solution to a ruby red hue validates the reduction of selenium ions into stable nanoparticles (Alagesan and Venugopal, 2019; Cittrarasu et al., 2021; Ahmed et al., 2025). Though enzymes have been exploited for the green synthesis of nanoparticles, there is paucity of reports on the exploitation of pectinase to produce nanoparticles generally, and SeNPs in particular. To the best of our knowledge, this report is the first to demonstrate the green synthesis of SeNPs using pectinase. Enzymes have capabilities to scavenge electrons on metal ions, to reduce them to zero-valent nanoparticles in the green synthesis of nanoparticles which is facilitated be electrostatic interactions (Elegbede and Lateef, 2021).

The UV-Vis absorption spectra of AFP-SeNPs exhibits a significant absorption peak at 268 nm, which acts as a definitive indicator of the effective synthesis of SeNPs (Fig. 4b). The absorption maximum ($\lambda_{max}$) aligns with previously documented values for biosynthesized SeNPs. Cavalu et al. (2018) documented 268 nm, Fouda et al. (2022) noted 266 nm, Afzal et al. (2021) also recorded 266 nm, Deepa et al. (2022) indicated 263 nm, while Ahmed et al. (2025) reported 295 nm as $\lambda_{max}$ values for synthesized SeNPs.

Fourier-transform infrared (FTIR) spectroscopy was conducted to examine the functional groups in both the pectinase enzyme and AFP-SeNPs to elucidate their interactions during nanoparticle formation. The comparative spectrum analysis indicates substantial structural alterations and functional group interactions between the native enzyme and the synthesized nanoparticles.

The FTIR spectra of pectinase exhibited major and minor peaks at 1077, 1636, 1964, 2120, 2176, and 3324 $cm^{-1}$. The peak at 1077 $cm^{-1}$ corresponds to C-O stretching, indicative of glycosidic linkages or polysaccharides, hence verifying the enzyme's glycoprotein origin. The amide I band at 1636 $cm^{-1}$, linked to C═O stretching in proteins, indicates the enzyme's secondary structure, predominantly consisting of α-helices and β-sheets. A prominent O-H/N-H stretching peak at 3324 $cm^{-1}$ signifies hydrogen bonding in proteins, underscoring the importance of hydroxyl and amine groups in maintaining structural integrity. Peaks in the 2105–2176 $cm^{-1}$ range may indicate C≡C stretching vibrations or interactions with selenium-organic compounds, but the 1964 $cm^{-1}$ peak likely signifies an overtone or combination band (Fig. 4c).

Following nanoparticles synthesis, the FTIR spectra of the selenium nanoparticles revealed peaks at 1636 $cm^{-1}$, 2105 $cm^{-1}$, and 3291 $cm^{-1}$. The amide I band at 1636 $cm^{-1}$ was preserved, demonstrating the sustained presence of proteins in the produced nanoparticles. The absence of the C-O stretching signal at 1077 $cm^{-1}$ indicates enzymatic interaction with selenium, which may modify or cleave glycosidic bonds. The existence of a specific 2105 $cm^{-1}$ signal indicates selenium-organic functional interactions, maybe resulting from Se-related bonding with biomolecules. The extensive O-H/N-H stretching band at 3291 $cm^{-1}$ persists, albeit with a minor shift relative to the natural enzyme, suggesting altered hydrogen bonding, probably attributable to SeNPs stabilization by proteins (Fig. 4c).

The FTIR analysis demonstrates that pectinase is essential for the reduction and stability of selenium nanoparticles. The persistence of the amide I band (1636 $cm^{-1}$) in both spectra corroborates the involvement of proteins in SeNPs production. The lack of the 1077 $cm^{-1}$ signal in the nanoparticle spectrum indicates structural alterations in the enzyme, potentially resulting from its interaction with selenium. The alterations in peak shifts and intensities within the 2105–2176 $cm^{-1}$ range signify robust bonding interactions between selenium and functional groups in pectinase. The displacement of the broad O-H/N-H peak indicates conformational alterations in the enzyme during nanoparticle synthesis, potentially enhancing the stability and bioactivity of the generated selenium nanoparticles. Thus, the fungal pectinase acted as both reducing and stabilizing agent in the one-pot production of selenium nanoparticles.

The TEM image elucidates the morphological properties of AFP-SeNPs (Fig. 4d). The nanoparticles are clearly discernible, with sizes varying from 50.97 nm to 98.43 nm. Measured sizes revealed distinct particles at 50.97 nm, 72.11 nm, 82.65 nm, and 98.43 nm, indicating a level of polydispersity. Furthermore, the SeNPs primarily have a nearly spherical form, but with minor morphological differences noted. The SAED pattern of the AFP-SeNPs displays luminous diffraction spots organized in concentric rings (Fig. 4e), affirming their polycrystalline characteristics implying an organized atomic structure.

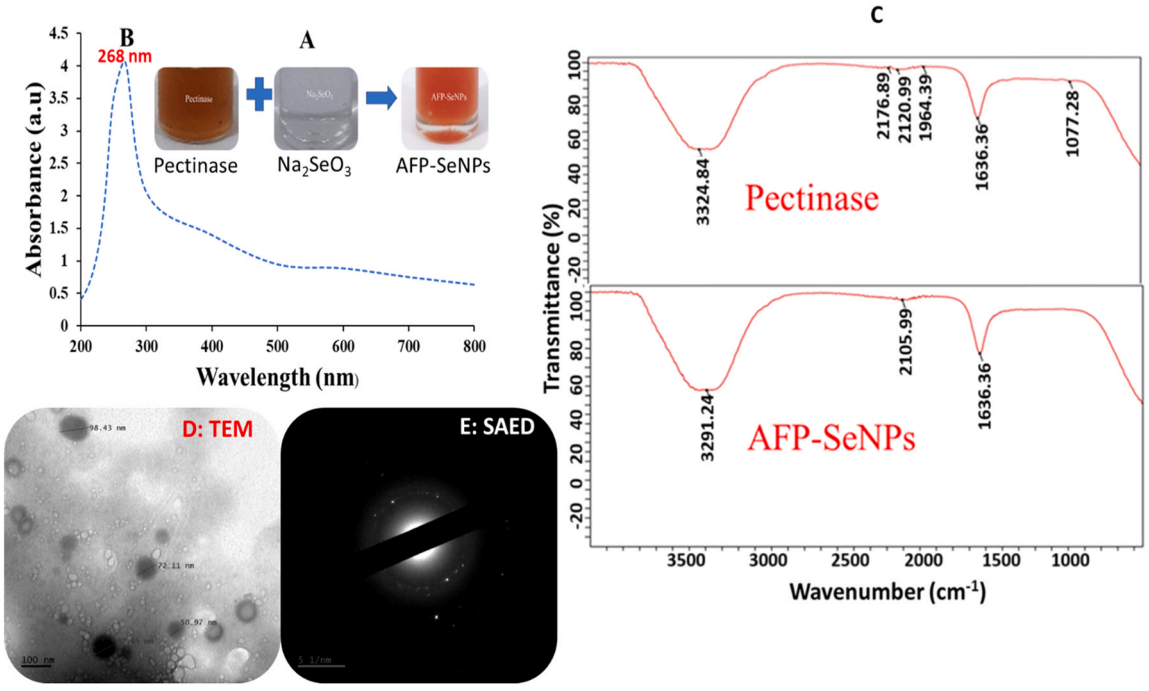



**Fig. 4.** Course of biosynthesis of AFP-SeNPs (A), UV-Vis spectrum of AFP-SeNPs (B), FTIR spectra of pectinase and AFP-SeNPs (C), TEM micrograph (D) and SAED pattern of AFP-SeNPs (E).

### 3.10. Antimicrobial activities of AFP-SeNPs

The results demonstrate a distinct concentration-dependent enhancement in antibacterial activities of AFP-SeNPs (Fig. 5). At a dose of 50 μg/ml, *S. enterica* exhibited the greatest susceptibility, with a zone of inhibition of 14.3 mm, whereas *K. oxytoca* showed no inhibition at this concentration. Conversely, *E. cloacae* consistently exhibited decreased susceptibility at all tested concentrations, signifying a comparative resistance to AFP-SeNPs relative to the other strains. With an increase in AFP-SeNPs concentration to 100 μg/ml, the antibacterial efficacy became more evident. *K. oxytoca*, which originally exhibited no inhibition, subsequently demonstrated a zone of inhibition of 20.7 mm, closely behind *S. enterica.* The change in susceptibility highlights the significance of dosage in the efficacy of AFP-SeNPs, indicating that elevated concentrations may improve the interaction between the nanoparticles and bacterial cells, resulting in enhanced antibacterial effects. Nonetheless, *E. cloacae* exhibited much lower susceptibility, which may be an indication of elevated resistance to antimicrobial agents. At elevated concentrations of 150 and 200 μg/ml, the bactericidal efficacy of AFP-SeNPs was significantly enhanced, with *K. oxytoca* exhibiting the greatest inhibition at 200 μg/ml, measuring 25.3 mm.

The antibacterial effectiveness of AFP-SeNPs against multiple bacterial species, such as *Salmonella enterica*, *Klebsiella oxytoca*, and *Enterobacter cloacae*, provides valuable insights into the prospective uses of selenium nanoparticles in addressing bacterial illnesses. AFP-SeNPs may specifically target bacterial mechanisms, possibly via the induction of oxidative stress or disruption of cellular integrity. However, the resistance demonstrated by *E. cloacae* at all tested concentrations prompts significant considerations for the formulation of effective antibacterial treatments. Studies have indicated that *E. cloacae* exhibits multiple resistance mechanisms, notably the synthesis of β-lactamases, which can deactivate numerous prevalent antibiotics (Afridi et al., 2021; Ganbold et al., 2023). Noteworthy is that *Enterobacter* species is a member of the notorious ESKAPE group with history of high drug-resistance and of high priority public health concerns (Ganbold et al., 2023). Also, both *Klebsiella oxytoca* and *Salmonella enterica* have been reported for their drug-resistance and the associated public health issues in causing nosocomial infections, and typhoid, respectively (Ikhimiukor et al., 2023; Jabeen et al., 2023).

The antibacterial activities of AFP-SeNPs show considerable promise for combating bacterial infections, especially those induced by *S. enterica* and *K. oxytoca*. The noted concentration-dependent enhancement of antibacterial activity highlights the significance of dosage in the efficacy of antimicrobial medicines. The resistance demonstrated by *E. cloacae* requires additional investigation into alternate techniques to improve antibacterial effectiveness against resistant strains.

The antifungal efficacy of AFP-SeNPs was excellent against the tested fungal species; *Aspergillus niger, Aspergillus flavus,* and *Penicillium* species with a total growth suppression (100 %) upon exposure to AFP-SeNPs, in contrast to abundance of growth in the control plates (Fig. 4c). These results align with other investigations that have reported significant inhibitory effects of selenium nanoparticles on fungi, underscoring their extensive antifungal capabilities (Abdelsalam, 2023; Serov, 2023; Gharieb, 2025). The antifungal actions of AFP-SeNPs may entail the loss of cell membrane integrity, interference with metabolic activities, and the induction of oxidative stress, resulting in fungal cell destruction (Vrandečić et al., 2020; Shahbaz et al., 2022). Oxidative stress occasioned by selenium nanoparticles can harm cellular constituents, such as lipids, proteins, and nucleic acids, ultimately leading to cell death (Huang et al., 2020; Varlamova, 2023; Sapronov, 2024).

The capacity of AFP-SeNPs to entirely suppress fungal proliferation is notably important due to the escalating risks that fungal infections present to food security and human health, and can therefore be applied in food preservation, agriculture, and the pharmaceutical sector. Fungal pathogens, particularly from the *Aspergillus* and *Penicillium* genera, are infamous for their capacity to deteriorate food products and induce diseases in humans and animals. Furthermore, the biogenic production of selenium nanoparticles provides a safer and more eco-friendly alternative to synthetic antimicrobial drugs, which frequently entail negative side effects and promote resistance development (Liang et al., 2022; Ušjak et al., 2022).

### 3.11. Anticoagulant activity of AFP-SeNPs

The anticoagulant properties of AFP-SeNPs assessed via macroscopic

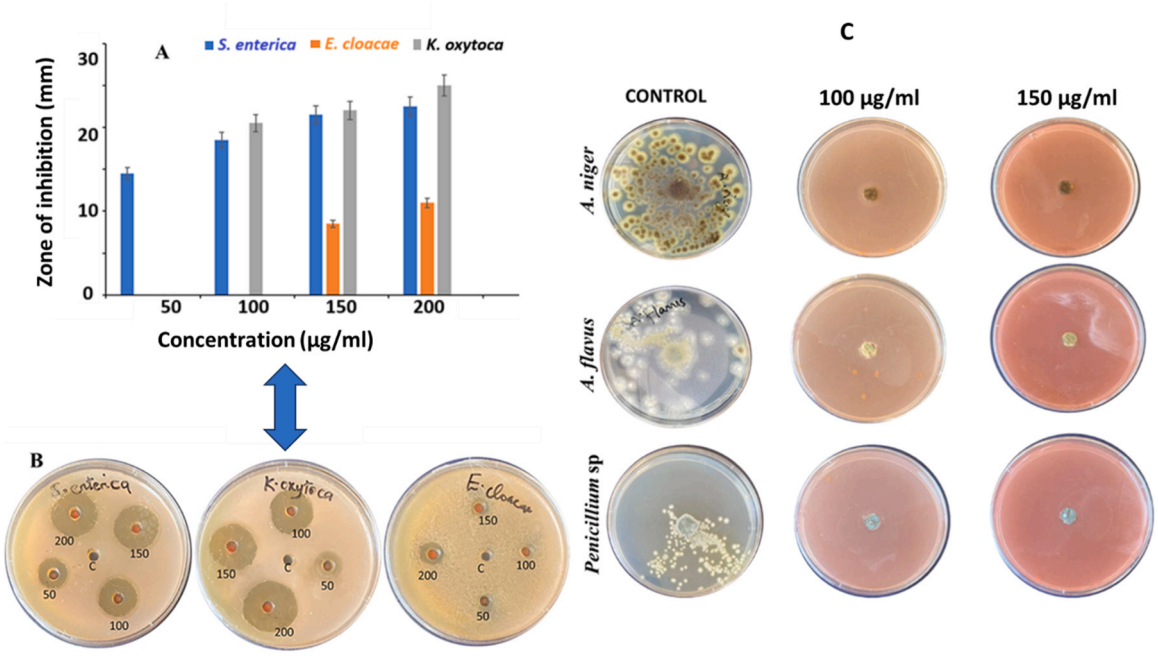



**Fig. 5.** Antibacterial activities of AFP-SeNPs against different pathogenic bacteria at different concentrations (A), representative images of zones of inhibition on Mueller-Hinton agar (B), and antifungal activities (C).

and microscopic evaluations showed its blood anticoagulation efficacy that prevented blood from clotting as obtained with positive control using EDTA (Fig. 6). Ethylenediaminetetraacetic acid (EDTA) is a recognized anticoagulant that chelates calcium ions, thus preventing clot formation. However, in the negative control where the blood was not exposed to any agent, total blood coagulation was obtained. These observations indicate that AFP-SeNPs may disrupt clot formation, possibly by modulating coagulation factors or calcium ion activity, rendering them as viable candidates for anticoagulant applications (Lateef et al., 2018; Van Paridon et al., 2022; Qin et al., 2023). Recently, some limited studies have reported the anticoagulant activities of SeNPs that were synthesized by plant extracts (Abdulmumini et al., 2025), citric acid (Alhawiti, 2024) and ascorbic acid (Siddique et al., 2024).

The microscopic evaluation further confirms the anticoagulant capabilities of AFP-SeNPs. The image of clotted blood was marked by closely clustered blood cells, signifying natural coagulation. Conversely, the EDTA-treated blood revealed a more distributed arrangement of blood cells. In the image illustrating blood treated with AFP-SeNPs, there was comparable distribution of blood cells, indicating successful coagulation prevention.

The mechanism by which AFP-SeNPs exert their anticoagulant effects may involve the modulation of key coagulation factors. The intrinsic pathway of coagulation involves several factors, including factor XII, XI, IX, and VIII, while the common pathway includes factors II, V, and X (Qin et al., 2023). The interaction of AFP-SeNPs with these factors could potentially disrupt the coagulation cascade, leading to the observed anticoagulant effect. Furthermore, the ability of AFP-SeNPs to maintain blood in a liquid state, as evidenced by the tube assay, suggests a significant alteration in the coagulation process, possibly by inhibiting thrombin generation or affecting platelet aggregation (Van Paridon et al., 2022). There are increasing evidences of the potencies of metal, alloy, and metal oxide nanoparticles such as Ag, Au, Ag-Au, $TiO_2$, Ti-Ag, and MgONPs as anticoagulant agents (Akinola et al., 2020; Azeez et al., 2020; Oladipo *et al.,* 2021; Akinola et al., 2022; Lateef et al., 2023) for potential applications in the management of blood coagulation disorders.

### 3.12. Antioxidant activities of AFP-SeNPs

The antioxidant activities of AFP-SeNPs, as illustrated in Fig. 7, exhibit considerable DPPH scavenging capability. The scavenging activity is significant at all evaluated concentrations, ranging from 35.9 % to 59.3 % at concentrations of 5–60 μg/ml. This underscores the free radical neutralization capability of AFP-SeNPs, indicating their efficacy in alleviating oxidative stress. SeNPs are well acknowledged for their antioxidant capabilities, and our findings further substantiate their potential biological applications. The observed activity can be ascribed to the distinctive physicochemical characteristics of AFP-SeNPs, which facilitate their effective interaction with and neutralization of reactive oxygen species. These findings correspond with prior research emphasizing the antioxidant capabilities of SeNPs produced via biological methods. El-Zayat et al. (2021) reported DPPH $IC_{50}$ of 0.296 mg/ml for SeNPs biosynthesized by *Ephedra aphylla*, while Abdel-Moneim et al. (2022) documented DPPH inhibition of 89 % using 500 μg/ml of SeNPs synthesized using *Spirulina platensis.* The inclusion of bioactive chemicals in AFP-SeNPs may augment their stability and antioxidant effectiveness. Due to their promising efficacy, AFP-SeNPs may be viable candidates for biological, pharmacological, and nutraceutical applications, especially in addressing oxidative stress-related illnesses.

### 3.13. Comparative analysis of production, optimization and applications of pectinases

In Table 6, we present an analysis of literature reports on the microbial production of pectinases in relation to the results that were obtained in the present study. Pectinases have been produced by different microorganisms that include fungi and bacteria on diverse sustainable substrates such as orange peel, banana peel, pawpaw peel, wheat bran,

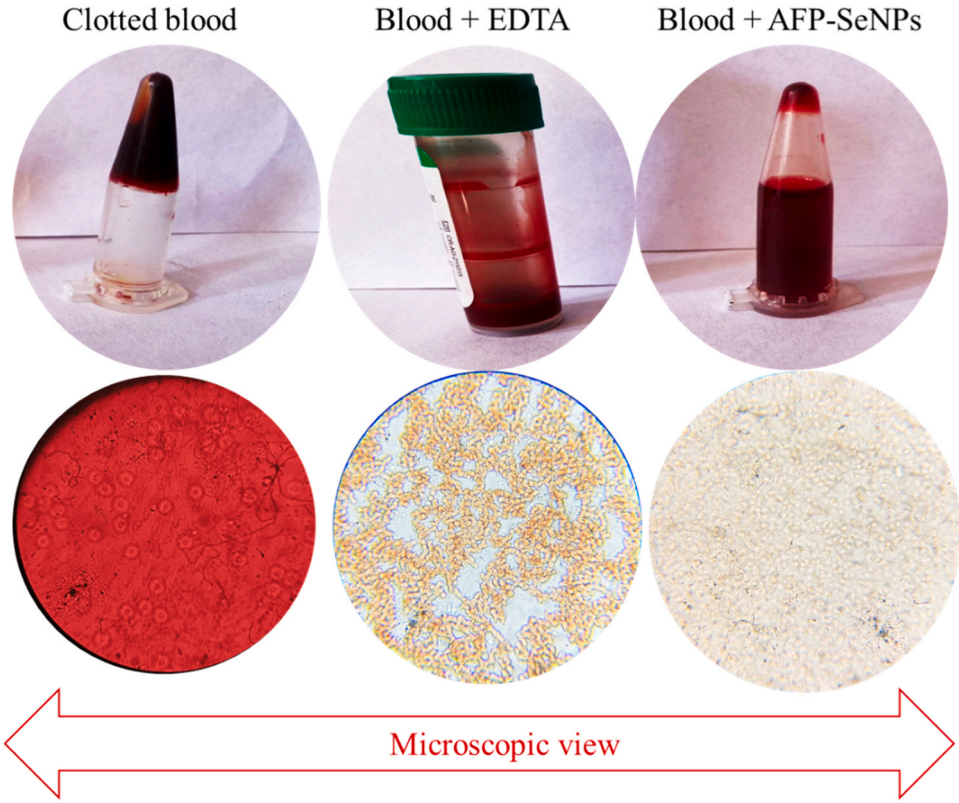



**Fig. 6.** Anticoagulant activity of AFP-SeNPs.

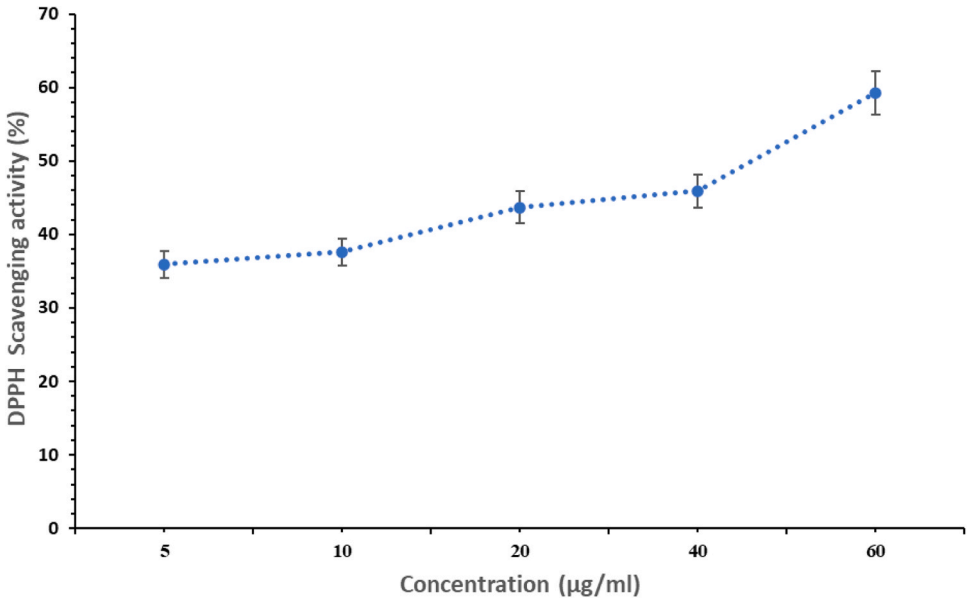



**Fig. 7.** DPPH scavenging activity of AFP-SeNPs.

**Table 6**
Comparative analysis of production, optimization and applications of microbial pectinases (2020–2025).

| Organism | Substrate used (concentration) | Optimization technique | Optimized yield | Yield improvement (fold) | Applications | References |
|---|---|---|---|---|---|---|
| *Penicillium chrysogenum* MF318506 | Orange peel (2.5 %) | Response Surface Methodology (RSM) | 1.292 U/ml | 6.04 | ___ | Ahmed and Abd El Rahman (2021) |
| *Enterobacter* sp. MF84 | Pectin | RSM | 14.16 U/mg | 12.21 | ____ | Abdollahzadeh et al. (2020) |
| *Bacillus* sp. BIOSMNF02 | *Citrus* pectin (1.5 % w/v) | RSM-D-optimal quadratic model | 6.51 U/ml | 9.30 | _____ | Satpathy et al. (2024) |
| *Glutamicibacter* sp. | Sugarcane bagasse | Plackett-Burman design (PBD) and RSM | 632.93 U/g | 10.0 | Demucilization of coffee bean, bioscouring of jute fibre | Arpana et al. (2024) |
| *Pseudomonas* sp. 13159349 | Wheat bran (20 g/l) | PBD and RSM | 315.65 U/g | 5.20 | ____ | Arpana et al. (2023) |
| *Piriformospora indica* | Sugar beet pulp (20 g/l) | RSM | 19.4 U/ml | 5.84 | ____ | Kiani et al. (2023) |
| *Aspergillus niger* AN07 | Papaya peel | RSM | 264.20 U/g | 5.40 | Pomegranate juice clarification | Patidar et al. (2020) |
| *Bacillus subtilis* BK−3 | *Citrus* peel | RSM | 43.46 IU/ml | 2.47 | Fruit juice clarification (black mulberry, apple, orange, and pineapple) | Prajapati et al. (2021) |
| *Streptomyces fumigatiscleroticus* VIT-ST4 | Orange pectin | Taguchi | 98.65 U/ml | 2.15 | Drug delivery and concrete crack healing | Govindaraji and Vuppu (2020) |
| *Yarrowia phangngaensis* | Banana peel | One variable at a time (OVAT) | 1.610 IU/ml | 2.0 | Apple juice clarification | Ezeh et al. (2023) |
| *Geotrichum candidum* AHC1 | Apple waste and halophytic biomass | Mutation | 12.03 IU/ml | 1.88 | ____ | Hanif et al. (2023) |
| *Aspergillus niger* ATCC 1640 | Satkara peel (8.4 g/l) | RSM, PBD | 0.6045 µmol/ml | 1.07 | ____ | Ahmed et al. (2021) |
| *Serratia marcescens* R5 | Pectin (1 %) | OVAT | 8.91 U/min | 1.65 | Clarification of apple, lemon, and mango juices | Haile et al. (2022) |
| *Aspergillus aculeatus* NEJC | Mango peel (1 %) | OVAT | 1360 U/ml | 5.18 | Clarification of cashew juice | Omeje et al. (2023) |
| *Aspergillus parvisclerotigenus* KX928754 | Apple pomace | OVAT | 1912 U/ml | 1.40 | ____ | Satapathy et al. (2021) |
| *Aspergillus niger* LFP−1 | *Citrus* pectin (2.5 %) | OVAT | 7.41 U/ml | 8.42 | ____ | Jalil et al. (2023) |
| *Aspergillus niger* | Pineapple peel (1 %) | - | 0.885 U/ml | - | Coconut extraction | Ajayi et al. (2021) |
| *Aspergillus flavus* | Lemon peel (40 g/l) | Central composite rotatable design | 3.68 U/ml | 6.94 | Grape juice clarification | Afzia et al. (2024) |
| *Streptomyces* sp | Pectin (1.5 %) | RSM | 2.79 U/ml | 6.80 | Dye decolourization | Shrestha et al. (2023) |
| *Aspergillus flavus* KW03 | Orange peel (2.5 g/l) | Taguchi | 921.3 U/ml | 4.98 | Orange juice clarification, biosynthesis of SeNPs with antimicrobial, antioxidant and anticoagulant activities | This study |

pineapple peel, sugar beet pulp, apple waste, and sugarcane bagasse. Also optimization regimen consisting of response surface methodology, Plackett-Burman design, one-variable-at-a-time, and Taguchi technique have enhanced enzyme yields (2.15–12.21-fold) with the highest pectinase activity of 1912 U/ml reported for *Aspergillus parvisclerotigenus* KX928754 that was grown on apple pomace (Satapathy et al. 2021). Also, mutagenesis involving the applications of UV radiation and ethidium bromide have been used to enhance microbial production of pectinase (Hanif et al., 2023). Comparatively, the optimum 921.3 U/ml obtained in the present study for *Aspergillus flavus* KW03 is in the top-tier of pectinase yield reported in the last 5 years.

Some applications that have been reported for the aforementioned pectinases are demucilization, bioscouring, juice clarification, and coconut extraction. Several studies did not report applications for their synthesized pectinases. Furthermore, there is paucity of its use to produce nanoparticles which is limited to the works of Zhang et al. (2017) to synthesize AuNPs. Therefore, our study has innovatively extended the biotechnological applications of pectinase, producing selenium nanoparticles for the first time with antimicrobial, antioxidant and anticoagulant activities for potential biomedical and environmental applications.

## 4. Conclusion

This research effectively utilized the Taguchi technique to enhance pectinase synthesis by a non-aflatoxigenic *Aspergillus flavus* KW03 grown on orange peel-based medium resulting in 397.7 % improvement in enzyme yield, culminating in a final activity of 921.3 U/ml. The enzyme clarified orange juice by 76.6 %, indicating its suitability as a clarifier in juice manufacturing. The pectinase is hereby reported for the green synthesis of selenium nanoparticles (AFP-SeNPs) for the first time, showcasing an environmentally sustainable and economical approach to nanoparticle fabrication. The ruby red AFP-SeNPs displayed maximum absorbance at 268 nm and demonstrated potent antimicrobial efficacies against bacteria (up to 25.3 mm) and fungi (100 %), with substantial antioxidant activity of 35.9–59.3 % using DPPH. Furthermore, SeNPs prevented coagulation of blood *in vitro* while protecting the integrity of RBC as seen under the microscope. These findings indicate that AFP-SeNPs may be viable candidate for use in biomedical, food, and environmental applications to reduce the scourge of microbial infections, oxidative stress by free radicals, and management of blood coagulation disorders. This study has further extended the sustainable and scalable enzyme-assisted nanoparticle fabrication by successfully synthesizing SeNPs using fungal pectinase. The multifunctionalities of pectinase herein reported add to the growing applications of pectinase in the food, environmental and health sectors.

## CRediT authorship contribution statement

**Abdulmumini Shakirat A:** Writing – original draft, Visualization, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Gueguim-Kana E.B:** Software, Resources, Formal analysis. **Lateef Agbaje:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Data curation, Conceptualization. **N Matyumza:** Software, Resources, Formal analysis, Data curation. **Beukes L.S:** Software, Resources, Formal analysis.

## Declaration of Competing Interest

Authors declare that they do not have conflict of interests

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## Data availability

Data will be made available on request.

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