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RESEARCH ARTICLE

Isolation, Characterization, and Optimization of Glucoamylase – Producing Bacteria from Fruit Waste Soil for Industrial Applications

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ABSTRACT: Glucoamylases are exo-amylases that play a crucial role in starch hydrolysis, converting it into glucose, a fundamental process in industries such as food, biofuels, bioplastics, and chemicals. Fruit waste soil, rich in organic matter, harbors diverse microbial communities capable of producing industrially significant enzymes. This study aimed to isolate, characterize, and optimize glucoamylase-producing bacteria from fruit waste soil collected from Tasawade MIDC, Karad. Four bacterial isolates (T2, T3, T4, T5) were screened for glucoamylase production using starch agar medium, with isolate T5 exhibiting the highest enzymatic activity. The enzyme production was optimized under varying conditions of pH, temperature, salt concentration, metal ions, and substrate concentration. Maximum enzyme activity was observed at pH 6, 25°C, 0.2 mg/ml salt concentration, and 2.5 mg/ml substrate concentration, with $MgCl_2$ (0.3 mg/ml) enhancing enzyme activity. Among the isolates, T5 demonstrated superior glucoamylase production (176.45 U/ml/min) and specific activity (309.56 U/mg). Morphological and biochemical characterization revealed that T5 was a Gram-positive, non-motile coccus, while T2 was a Gram-positive, motile, spore-forming rod. The stability and efficiency of the isolated glucoamylase under diverse conditions suggest its potential for industrial applications. Further research on large-scale production, purification, and kinetic studies could enhance its utility in starch processing, biofuel production, and other biotechnological industries. This study highlights the significance of microbial enzymes from organic waste sources as sustainable and cost-effective alternatives for industrial processes.

Keywords: Glucoamylase, Fruit waste soil, Starch hydrolysis, Enzyme optimization, Bacterial isolation, Industrial applications

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1. INTRODUCTION

Enzymes are protein-based biological catalysts that play an essential role in accelerating biochemical reactions necessary for cellular metabolism [1]. These biomolecules are synthesized by living cells and have been harnessed for numerous industrial applications due to their specificity, efficiency, and ability to function under mild conditions [2]. Among the commercially significant enzymes, those derived from microorganisms are particularly valuable due to their scalability and adaptability to industrial processes [3]. Amylases, a major class of hydrolytic enzymes, are pivotal in breaking down complex polysaccharides such as starch into simpler sugars like glucose and fructose [4, 5]. These enzymes are extensively utilized in starch processing industries, where their ability to cleave glycosidic bonds is crucial for producing sweeteners, syrups, and fermentation substrates [6, 7].

Glucoamylase (GA, α-1,4-glucan glucohydrolase) is an exo-acting amylase that sequentially hydrolyzes α-1,4 and α-1,6 glycosidic linkages in starch, yielding glucose as the sole end product [8, 9]. Unlike other amylases, glucoamylase can theoretically achieve complete starch conversion into

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glucose, though the cleavage of α-1,6 linkages occurs at a significantly slower rate compared to α-1,4 bonds [10,11]. This enzyme is naturally produced by various organisms, including plants, animals, fungi, and bacteria, with microbial sources being the most preferred for industrial applications due to their high yield and ease of genetic manipulation [9].

Fungal glucoamylases, particularly from *Aspergillus and Rhizopus* species, have traditionally dominated industrial processes, but their requirement for acidic pH and susceptibility to denaturation pose limitations [12-14]. Bacterial glucoamylases, on the other hand, offer advantages such as broader pH stability, higher thermotolerance, and compatibility with diverse industrial conditions, making them an attractive alternative [12]. Enzymatic starch hydrolysis is favored over chemical methods due to its specificity, reduced byproduct formation, and lower energy consumption [13]. However, the economic feasibility of enzyme-based processes depends on optimizing production yields and enhancing catalytic efficiency. Bacterial strains capable of producing glucoamylase under cost-effective conditions are thus of great interest [14].

This study focuses on isolating glucoamylase-producing bacteria from fruit waste soil—a nutrient-rich environment that supports diverse microbial communities. The rationale behind using fruit waste soil lies in its high organic content, which promotes the growth of microorganisms with hydrolytic enzyme capabilities [15]. The research objectives include the isolation and biochemical characterization of glucoamylase-producing bacteria, optimization of enzyme production conditions, and preliminary assessment of kinetic properties to evaluate industrial applicability [16, 17].

The novelty of this work lies in the exploration of fruit waste soil as an underexplored microbial niche for glucoamylase production. While previous studies have predominantly focused on fungal sources or conventional bacterial strains, this research identifies and characterizes bacterial isolates from an organic waste substrate, offering a sustainable and economical alternative for enzyme production.

Furthermore, the optimization of physicochemical parameters (pH, temperature, metal ions, and substrate concentration) provides insights into enhancing enzyme activity under industrially relevant conditions. By demonstrating the potential of these bacterial isolates, this study contributes to the development of greener and more efficient bioprocesses for starch hydrolysis, aligning with global trends toward sustainable industrial practices [18-22].

The findings of this research have significant implications for industries reliant on starch conversion, including food processing, biofuel production, and biodegradable plastics. By reducing dependency on traditional fungal enzymes and leveraging waste-derived microbial resources, this study advances the field of industrial enzymology while promoting environmental sustainability. Future directions include scaling up enzyme production, structural characterization, and genetic engineering to further improve catalytic performance for commercial applications.

2. EXPERIMENTAL DETAILS

2.1. Sample Collection and Preparation

Fruit waste soil samples were collected from an industrial site in Tasawade MIDC, Karad, Maharashtra, India. Sampling was performed at a depth of 2–4 cm to ensure the collection of microbial populations actively involved in organic matter decomposition. Four replicate samples were aseptically collected in sterile polyethylene bags to prevent contamination and moisture loss. The samples were transported to the laboratory under cool conditions and stored at 4°C until further processing to maintain microbial viability. Prior to analysis, soil samples were homogenized and sieved (2 mm mesh) to remove large debris and ensure uniformity.

2.2. Screening and Isolation of Glucoamylase-Producing Bacteria

Primary screening for glucoamylase-producing bacteria was conducted using starch agar plates (composition: 1% soluble starch, 0.5% peptone, 0.5% yeast extract, 0.1% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, and 1.5% agar, pH 7.0). Soil suspensions were prepared in sterile saline (0.85% NaCl) and serially diluted (10^{-1} to 10^{-6}). Aliquots (100 µL) from appropriate dilutions were spread-plated onto starch agar and incubated at 37°C for 24 h. Colonies exhibiting hydrolytic activity were identified by flooding the plates with Gram's iodine solution; clear zones around colonies indicated starch degradation [22]. Putative glucoamylase-producing isolates were purified via quadrant streaking on fresh starch agar plates. Pure cultures were maintained on starch agar slants at 4°C for short-term storage and in 20% glycerol at −80°C for long-term preservation.

2.3. Characterization of Bacterial Isolates

The selected isolates were characterized based on cultural, morphological, and biochemical properties. Colony morphology (size, shape, color, elevation, and margin) was recorded after 24 h of growth on starch agar. Gram staining was performed using the standard protocol [11], and motility was assessed via hanging drop microscopy. Biochemical tests included catalase activity (3% H_2O_2), oxidase (Kovacs' reagent), Voges-Proskauer (VP test), and carbohydrate fermentation (phenol red broth with arabinose and mannitol) [11, 20].

2.4. Production of Glucoamylase

Glucoamylase production was carried out in 150 mL of optimized liquid medium (1% starch, 0.5% peptone, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, pH 6.0) inoculated with a 1% (v/v) overnight culture of selected isolates. Flasks were incubated at 37°C with shaking (150 rpm) for 48 h. The

culture broth was centrifuged (10,000 ×g, 15 min, 4°C), and the cell-free supernatant was retained as the crude enzyme extract [20]. The supernatant was stored at −20°C to preserve enzyme activity until further analysis.

2.5. Glucoamylase Assay

Enzyme activity was determined using the 3,5-dinitrosalicylic acid (DNSA) method [22]. The reaction mixture contained 0.5 mL of 1% (w/v) soluble starch in 0.1 M sodium acetate buffer (pH 6.0) and 0.5 mL of crude enzyme. After incubation at 37°C for 10 min, the reaction was terminated by adding 1 mL DNSA reagent, followed by boiling for 5 min. The absorbance of the resulting reducing sugars (maltose equivalents) was measured at 540 nm. A maltose standard curve (0.1–1.0 mg/mL) was used to quantify glucose liberation.

Protein concentration was determined via the Biuret method using bovine serum albumin (BSA) as the standard [21]. Specific activity was expressed as units per milligram of protein (U/mg), where one unit (U) of glucoamylase activity was defined as the amount of enzyme liberating 1 µmol of glucose per minute under assay conditions.

2.6. Kinetic Characterization of Glucoamylase

2.6.1. Effect of pH on Enzyme Activity

The pH optimum was determined by assaying enzyme activity in buffers of varying pH (5.0–9.0): sodium acetate (pH 5.0–6.0), phosphate (pH 7.0), Tris-HCl (pH 8.0), and glycine-NaOH (pH 9.0). Reactions were conducted at 37°C, and residual activity was measured relative to the optimal pH.

2.6.2. Effect of Temperature on Enzyme Activity

Thermal stability was assessed by incubating the enzyme at temperatures ranging from 4°C to 70°C for 30 min before measuring residual activity. The optimum temperature was identified as the point of maximum activity.

2.6.3. Effect of Metal Ions and Inhibitors

The influence of metal ions (Ca^{2+}, Mg^{2+}, Na^{+}, Hg^{2+}) was evaluated by pre-incubating the enzyme with 1 mM solutions of $CaCl_2$, $MgCl_2$, NaCl, and $HgCl_2$ for 10 min. Relative activity was compared to a control (no metal ions).

2.6.4. Effect of Salt Concentration

Enzyme activity was measured at NaCl concentrations ranging from 0.1 to 0.5 mg/mL to determine halotolerance.

2.6.5. Effect of Substrate Concentration

Kinetic parameters (Km and Vmax) were determined by varying starch concentrations (0.5–5.0 mg/mL). Data were analyzed using Lineweaver-Burk plots.

3. RESULTS AND DISCUSSION

3.1. Isolation and Screening of Glucoamylase-Producing Bacteria

Fruit waste soil collected from Tasawade MIDC, Karad, was found to harbor diverse bacterial populations capable of starch hydrolysis. Initial screening on starch agar plates revealed four distinct bacterial isolates (T2, T3, T4, T5) that exhibited clear zones of hydrolysis upon iodine staining, indicating glucoamylase activity. Isolate T1 did not show any hydrolytic activity and was excluded from further analysis. The zone-to-colony size ratio (S/R ratio) was used as a quantitative measure of enzyme production efficiency (Table 1). Among the isolates, T5 demonstrated the highest S/R ratio (0.73), suggesting superior glucoamylase secretion compared to T2 (0.50), T3 (0.50), and T4 (0.55). These findings align with previous studies that utilized S/R ratios as a reliable indicator of amylolytic potential in microbial isolates [22-26].

Table 1. Isolate codes with their S/R ratios.

Isolate Code	Colony Size (mm)	Zone diameter (mm)	S/R ratio
T2	3	1.5	0.50
T3	2	1.0	0.50
T4	2	1.1	0.55
T5	3	2.2	0.73

3.2. Morphological and Biochemical Characterization

3.2.1. Colony Characters, Gram nature and Morphology of the Isolates

The size of the colonies of isolates namely are T2 was 1.5 mm, T3 was 1.0 mm, T4 was 1.1 mm and T5 was 2.2 mm in diameter. Colonies of the two isolates namely T3 and T4 were circular in shape, while of the isolate T2 and T5 were irregular. Colonies of all isolate were white in color. T2 was transparent and T3, T4, T5 were opaque. All isolates were moist. Colonies of the isolate namely T3, T4 showed entire margin while of T2 and T5 showed undulate in margin. Colonies of all isolates were flat elevation. The isolates namely T2 were gram positive short rods while T3, T4, T5 were gram positive cocci. All the isolates were non motile, expect T2 was motile in nature.

3.2.2. Biochemical Characterization of Isolates

The biochemical test for the isolate T2 shows as Voges

Proskauer test is negative, endospore staining is Positive, 6.5% NaCl growth test is negative, Phenol red arabinose broth test is negative**.** The Isolate T3, T4, T5 as shows as catalase Test is positive, mannitol fermentation test is negative.

3.3. Glucoamylase Production and Specific Activity

Quantitative analysis of glucoamylase activity revealed significant variation among isolates (Table 2). T5 exhibited the highest enzyme activity (176.45 U/mL/min) and specific activity (309.56 U/mg), outperforming T2 (82.96 U/mL/min), T3 (62.32 U/mL/min), and T4 (57.80 U/mL/min). The superior performance of T5 may be attributed to its efficient secretory machinery or genetic predisposition for high-yield enzyme synthesis [14]. These results corroborate earlier findings that certain bacterial strains isolated from organic waste exhibit enhanced glucoamylase production due to adaptive metabolic pathways [16].

Table 2. Units of Glucoamylase and Specific activity Produced from Isolates.

Isolates	T2	T3	T4	T5
Units /ml/min	82.96	62.32	57.80	176.45
Specific activity	145.54	109.33	109.40	309.56

3.4. Kinetic Characterization of Glucoamylase from T5

3.4.1. pH Optimum and Stability

The enzyme displayed maximal activity at pH 6.0 (Figure 1), with a sharp decline in activity at alkaline pH (8.0–9.0). This pH profile is consistent with bacterial glucoamylases from Bacillus licheniformis, which also exhibit optimal activity under slightly acidic conditions [23]. However, fungal glucoamylases typically favor more acidic pH (4.0–5.0) [24], underscoring the advantage of bacterial enzymes for neutral-pH industrial processes.

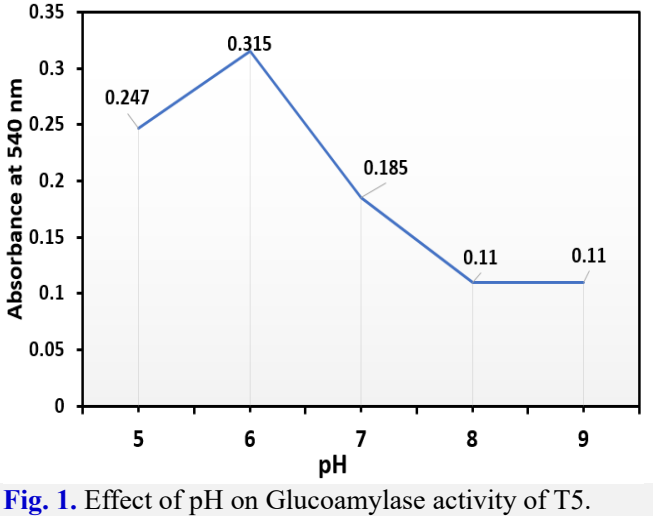

Fig. 1. Effect of pH on Glucoamylase activity of T5.

3.4.2. Temperature Optimum and Thermostability

The glucoamylase showed peak activity at 25°C (Figure 2), a lower optimum temperature compared to thermophilic bacterial enzymes (e.g., 45°C–60°C) [25]. This suggests mesophilic adaptation, possibly due to the temperate soil environment of origin. Notably, activity dropped sharply above 37°C, indicating limited thermostability—a trait that may require protein engineering for high-temperature applications [26].

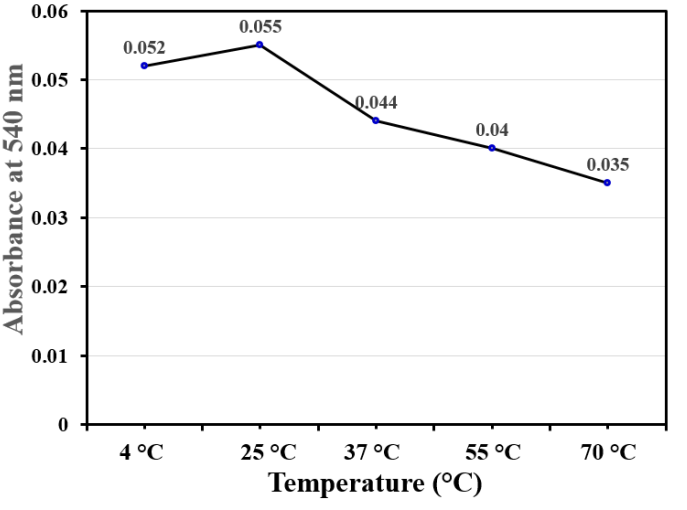

Fig. 2. Effect of Temperature on Glucoamylase activity of T5.

3.4.3. Metal Ion Effects

Mg^{2+} (0.3 mg/mL) enhanced enzyme activity by 20% (Figure 3), likely due to structural stabilization of the active site. In contrast, Hg^{2+} caused complete inhibition, a common observation due to sulfhydryl group disruption in enzyme structures [22]. NaCl (0.9 mM) had negligible effects, suggesting halotolerance up to 0.2 mg/mL (Figure 4).

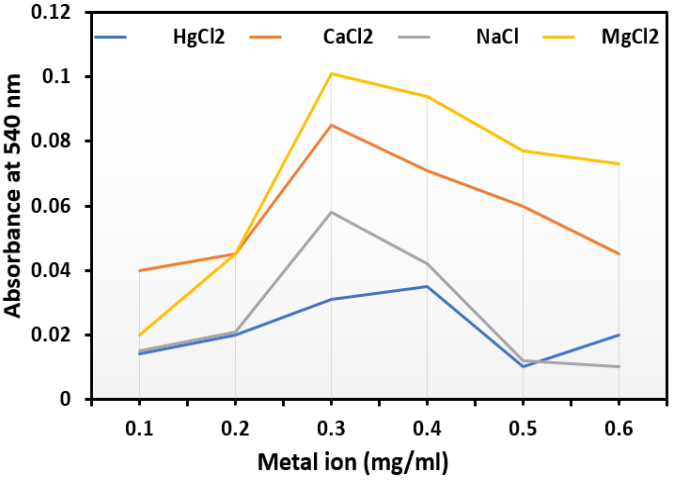

Fig. 3. Effect of Metal ion on Glucoamylase activity of T5.

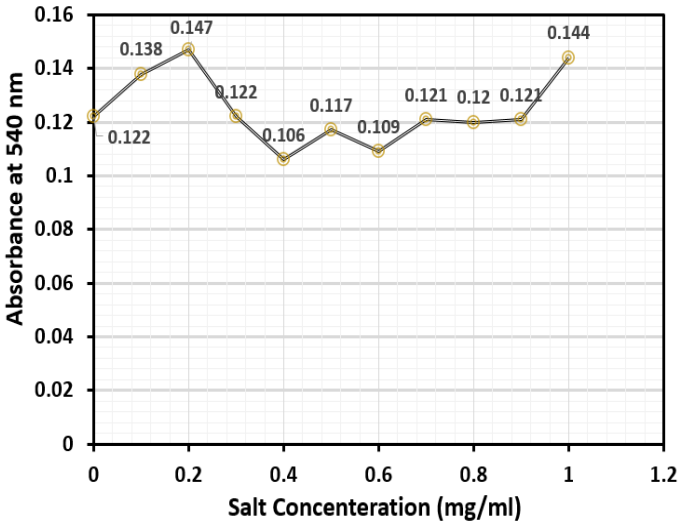

Fig. 4. Effect of Salt Concentration on Glucoamylase activity of T5.

3.4.4. Substrate Kinetics

The enzyme followed Michaelis-Menten kinetics, with Km = 0.7 mM and Vmax = 0.082 µM/min (Figure 5). The low Km indicates high substrate affinity, while the saturation at 2.5 mg/mL starch reflects limited catalytic turnover compared to fungal glucoamylases [12]. This kinetic profile may be optimized via fermentation conditions or strain improvement [14]. The isolate T5 demonstrates promising traits for starch-processing industries, particularly in low-temperature applications like food-grade glucose syrup production. Its neutral pH optimum and salt tolerance further broaden its utility in fermentation processes [18]. However, scalability challenges—such as moderate thermostability and substrate saturation—must be addressed through immobilization or genetic modification [19].

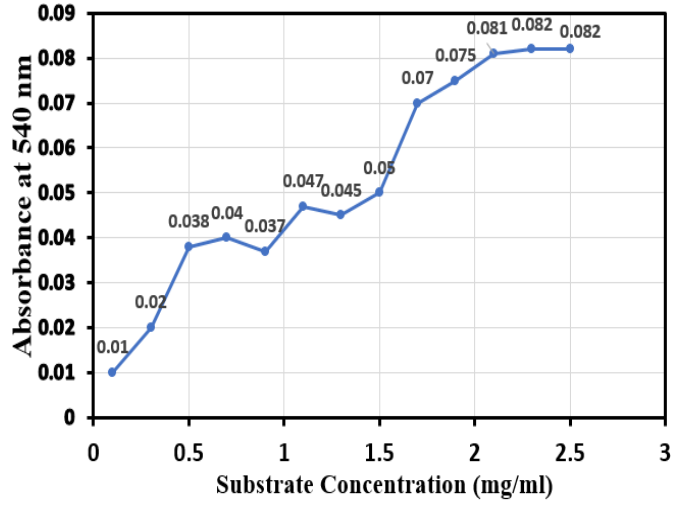

Fig. 5. Effect of Substrate Concentration on Glucoamylase activity of T5.

While fungal glucoamylases dominate industrial use, bacterial alternatives like T5 offer advantages in pH flexibility and reduced byproduct formation [13]. The observed Km (0.7 mM) is lower than reported for Aspergillus niger (1.2 mM) [12], suggesting superior substrate binding. However, the lower Vmax highlights a trade-off between affinity and catalytic efficiency. The study successfully identified T5 as a high-yield glucoamylase producer with unique kinetic properties. Future work should explore strain engineering for enhanced thermostability and large-scale production feasibility. The findings underscore the potential of fruit waste soil as an underexplored resource for industrial enzyme discovery.

4. CONCLUSION

This study successfully isolated and characterized glucoamylase-producing bacteria from fruit waste soil, demonstrating their potential for industrial applications. Among the four bacterial isolates screened, T5 exhibited the highest glucoamylase activity (176.45 U/ml/min) and specific activity (309.56 U/mg), making it the most promising candidate for further investigation. The optimization studies revealed that the enzyme functioned optimally at pH 6, 25°C, with $MgCl_2$ (0.3 mg/ml) enhancing its activity, while a salt concentration of 0.2 mg/ml and substrate concentration of 2.5 mg/ml were found to be ideal for maximum catalytic efficiency. The morphological and biochemical characterization of the isolates provided insights into their taxonomic classification, with T5 identified as a Gram-positive, non-motile coccus, contrasting with T2, which was a motile, spore-forming rod. The enzyme's stability under varying environmental conditions suggests its robustness, making it suitable for industrial processes that require consistent performance under different operational parameters. The findings of this study contribute to the growing body of research on microbial enzymes as sustainable alternatives to conventional chemical catalysts. The use of fruit waste soil as a microbial source not only provides an eco-friendly approach to enzyme production but also aligns with waste valorization strategies. Future research should focus on scaling up enzyme production, purification, and immobilization techniques to enhance yield and stability. Additionally, exploring genetic modifications or strain improvement strategies could further optimize enzyme characteristics for specific industrial needs. Given the increasing demand for efficient and eco-friendly biocatalysts in food processing, biofuel production, and other biotechnological applications, this study underscores the importance of exploring underutilized microbial resources. The glucoamylase-producing bacteria isolated in this research present a viable solution for sustainable industrial enzyme production, reducing reliance on synthetic processes and contributing to greener biotechnology practices. Further investigations into large-scale applications and process integration will be essential to fully harness their industrial

potential.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

Ethical Compliance Statement

This is an observational study. The Institutional Ethics Committee of Yashwantrao Chavan College of Science, Karad, has confirmed that no ethical approval is required. This research article does not involve any studies with human participants or animals performed by the authors.

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Not applicable

Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

Darshan Hosmath: Conceptualization, Methodology, Investigation, Writing – Original Draft. **Jaysing Patil:** Supervision, Validation, Writing – Review & Editing.

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