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Valorisation of Red Banana (*Musa acuminata*) Peel as a Source of Bioactive Natural Pigment

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A B S T R A C T

Fruit processing industries produce an enormous amount of peel waste every year. But these by-products are still not used by industry despite the high content of bioactive pigments and antimicrobial compounds provided by the peel. The present work aims to extract, characterize and analyse the functional properties of the natural pigment of red banana (*Musa acuminata*) peel and evaluate its stability in dairy-based and juice-based food systems. The pigment was extracted from fresh red banana peels using food grade ethanol as solvent. The crude peel was concentrated through the rotary evaporation process and converted to a dry powder by lyophilisation to obtain a powder for analysis and food application. The pigment was chemically characterized by Fourier Transform Infrared spectroscopy (FTIR) that found distinct functional groups of anthocyanins and phenolics that were used to identify and verify the pigment. Antibacterial activity was measured with *Staphylococcus spp, Bacillus spp, Escherichia coli, Proteus spp, and Clostridium spp* by agar well diffusion method and the diameter of inhibition zone was compared to standard antibiotic control. The pigment was then applied to a dairy, juice-based food model system and its stability was evaluated under different temperature and light conditions by measuring colour intensity and absorbance at various intervals. The stability tests showed remarkable antibacterial activity in the pigment for all species. In the stability studies, colour was found stable in refrigerated conditions. The discovery of red banana peel as a bioactive natural pigment with functional antimicrobial properties and acceptable stability to use in juice-based food products, also proved reliable towards probiotic count further reinforces the attractiveness of fruit processing products as clean-label natural colourants.

Introduction

The main reasons behind the global shift away from synthetic food and textile colourants have been concerns of consumers and regulators about their long term safety and increasing demand for sustainable, plant-based alternatives. Anthocyanins — water-soluble flavonoid pigments responsible for red, purple, and blue

colouration in numerous fruits, flowers, and vegetative tissues — have attracted sustained research interest due to their dual function as natural colourants and bioactive compounds, including well-documented antioxidant, antiviral, anticancer, antibacterial, and anti-inflammatory properties Yang *et al.,* 2015. The most extensively grown fruit crop in the world is the banana (*Musa spp*.). On ripening, however, the majority of commercial cultivars have yellow peel colouration, a characteristic associated with carotenoid rather than anthocyanin accumulation (Fu *et al.,* 2018). On the other hand, only a few cultivars have characteristic red peel colour. Fu *et al.,* (2018) showed that red banana peel coloration is mostly determined by the presence of anthocyanin-containing epidermal cell layers using UPLC-PDA-QTOF-MS and HPLC-PDA techniques. They found that the pigments unique to the red peel, presumably responsible for the characteristic color, are rutinoside derivatives of cyanidin, peonidin, petunidin and malvidin. However, Yang *et al.,* (2015) mentioned that there is still a lack of comprehensive understanding of the distribution of pigments in banana fruit, and there is still no complete and generalizable pigment profile for red- and yellow-peeled banana cultivars.

Red banana peel is an under-exploited by-product of banana processing and consumption from a valorisation point of view. Rosalin *et al.,* (2022) showed that the total anthocyanin content of the aqueous extraction of red banana peel was 55,139 mg/L, while the total anthocyanin content extracted with ethanol as the solvent was only 5,038 mg/L. This shows a significant solvent dependent extraction efficiency, with water as the preferred low cost, food safe extraction medium. Likewise, Rosalina *et al.,* (2022) extracted anthocyanins from red banana peel and bracts by maceration method with citric acid-acidified water and obtained yields of 110.27 µg/g and 114.26 µg/g fresh weight, respectively, which proves that both tissues are potential source of pigments.

However, these same studies also highlight a critical barrier to industrial application: pigment instability. Rosalina *et al.,* (2022) concluded that temperature treatments at 35°C and 50°C resulted in a significantly higher rate of pigment absorbance decline than treatments at 30°C and 40°C, while They also showed that irradiation with UV lamps, sunlight, and standard incandescent lighting accelerated anthocyanin degradation compared to exposure to lower-intensity light. Taken together, these data, suggest that the anthocyanins derived from red banana are highly sensitive to thermal and photolytic degradation. This limitation has to be considered before large scale application as a food or packaging colourant.

The present literature tends to support the statement that red banana peel is a genuine and unexploited source of anthocyanins and also points out a number of unresolved gaps. Firstly, systematic optimisation of extraction parameters such as solvent polarity, pH, temperature and solid to liquid ratio has not been extensively studied for red banana peel in comparison with other anthocyanin sources such as grape skin, red cabbage and roselle calyces. Secondly, although qualitative observations on the temperature and light induced deterioration have been reported (Rosalina *et al.,* 2022), quantitative degradation kinetics and pigment stabilization techniques (e.g. encapsulation, co-pigmentation and pH buffering) have not been extensively explored for this substrate. Third, it is unclear how traditional maceration compares to more environmentally friendly or effective extraction techniques for red banana peel specifically. Hence, the objective of this research is to investigate the extraction of pigments from red banana peels for aim such as optimizing extraction parameters and pigment stability under different pH, temperature, and light conditions This work provides a systematic characterisation of extraction efficiency and pigment behaviour, contributing to fundamental understanding of *Musa-derived anthocyanins* and the practical development of red banana peel as a feasible, low-cost, sustainable natural colourant for the food, packaging and nutraceutical sectors.

Materials and Methods

The fresh (*Musa acuminata / Musa sapientum L. var. Rubra*) peels were purchased from a local fruit market in Bangalore, Karnataka, India. Ethanol (Food-grade), hydrochloric acid (HCl), Mueller–Hinton agar, nutrient broth, and all other chemicals and reagents were of high-quality analytical grade and were procured from Hi Media Laboratories Private Limited. Fresh milk (for yoghurt preparation) and commercial fruit juice were obtained from a local supermarket. All the experiments were carried out with de-ionized water.

Plant Material and Pre-treatment

Fresh red banana (*Musa acuminata / Musa sapientum L. var. Rubra*) peels were obtained, washed with distilled

water to remove surface debris, and cut manually into small pieces (~1–2 cm2).

Peels can be used fresh or can be oven-dried at 40–50°C until constant weight to avoid the microbial spoilage and degradation of pigment prior to extraction and then ground to fine powder using laboratory blender following maceration approach (Rosalin *et al.,* 2022**)**

Extraction of pigments from plants

Take 10g or 20g fresh red banana peel, sliced. Extraction solvent: 70% ethanol in water, pH adjusted to 3.5 with citric or hydrochloric acid. Mix the plant material and the solvent at about 1:10 solid to liquid (w/v).

Remove the mixture for 60 min to 24 h (depending on the extraction condition tested) under continuous stirring (magnetic stirrer, ~150–350 rpm) at room temperature protected from light (wrap the vessel in aluminum foil or carry out extraction in a darkened room) (Rosalina *et al.,* 2022)

The extract should be filtered under vacuum through Whatman No. 1 filter paper (or several layers of muslin cloth and then filter paper) in a Büchner funnel to remove solid residue.

Save the filtrate (crude pigment extract) for concentration by rotary evaporation. In case of non-immediate processing, store the filtrate at 4 °C in the dark for no longer than 24 h to avoid the degradation of the anthocyanins.

Concentration using rotary evaporation

Filter the crude extract and pass it through a round bottom flask attached to a rotary evaporator. The extract was concentrated under reduced pressure at a low bath temperature (35-45°C) to avoid thermal degradation of the heat-sensitive anthocyanin pigments, which is a common practice in anthocyanin extraction (Lestario *et al.,* 2014).

Evaporate until most of the solvent is gone, leaving a pigmented concentrated extract. The concentrated extract can be further dried in a vacuum oven at low temperature or freeze-dried (lyophilisation). The concentrated extract was stored in an amber (light-protected) container at −18 to 4°C until later analysis (Rosalina *et al.,* 2022).

Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was used to analyse the functional group composition of the red banana peel pigment extract. The lyophilised (freeze-dried) pigment powder was analysed using a Perkin Elmer Spectrum FTIR spectrometer (Spectrum IR software, version 10.7.2) with a Universal Attenuated Total Reflectance (UATR) accessory. A small amount of powder was placed directly on the diamond ATR crystal and good contact was achieved by the pressure clamp of the instrument. Spectra were listed in the wavenumber range of 4000–400 cm-1. The transmittance (%T) and absorbance (A) spectra were analysed by the instrument's automated peak-picking function to identify major absorption bands. Peak assignments were made by comparison with characteristic functional group frequencies reported in FTIR literature for anthocyanins and plant phenolics (Delgado *et al.,* 2000 & Gong *et al.,* 2024).

DPPH free radical scavenging activity assay

The antioxidant activity of the pigment was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay as described by Halim *et al.,* (2017) with some modifications. One millilitre of pigment suspension was added to 0.8 mL of 0.2 mM DPPH solution in a test tube, shaken well and kept in the dark for 30 min at room temperature. The optical absorbance of the reaction mixture was read at 517 nm using a visible-wavelength spectrophotometer, with methanol as the blank. The control was prepared by replacing the sample with 1 mL of methanol and the same procedure was followed. The percent of radical scavenging activity (RSA) was calculated as follows: RSA (%) = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100. The antioxidant activity was evaluated by IC50 (concentration of the sample to scavenge 50% of DPPH free radical.

Antimicrobial studies of the extracted pigment: Agar Well Diffusion Method

Preparation of Test Extract Solutions

The freeze-dried red banana peel pigment powder was reconstituted in DMSO to prepare a stock solution (e.g., 100 mg/mL), from which a dilution series was prepared

for concentration-dependent testing (Salmon *et al.,* 2025).

Test Microorganisms and Standardisation

Five bacterial strains were tested: *Bacillus cereus*, *Clostridium* spp., *Proteus* spp., *Pseudomonas* spp., and *Escherichia coli*. Aerobic/facultative organisms such as *B. cereus*, *Proteus* spp., *Pseudomonas* spp., *and E. coli* were revived on nutrient agar at 37°C for 18–24 h. *Clostridium spp***.** were revived on Reinforced Clostridial Medium under anaerobic conditions (in an anaerobic jar with a gas-generating sachet). Each culture was standardised to 1.5 × 10^8 CFU/mL using sterile saline/distilled water prior to testing.

Agar Well Diffusion Procedure

The antimicrobial activity of the red banana (*Musa acuminata*) peel pigment was evaluated against five pathogenic microorganisms, *Escherichia coli, Proteus spp., Pseudomonas spp., Clostridium spp., and Bacillus spp*. using the agar well diffusion method as described by Devi *et al.,* (2024). The extracted red banana peel pigment was suspended in dimethyl sulfoxide (DMSO) at a stock concentration of 1 mg/mL to prepare the test solution. DMSO was selected as the solvent for pigment suspension due to its established ability to dissolve hydrophobic bioactive compounds, its negligible antimicrobial activity at the volumes used, and its wide acceptance as a vehicle solvent in antimicrobial assays (Ji K. and Kim Y. T. 2019). From this stock solution, three different volumes were loaded into the agar wells for antimicrobial evaluation. For *Escherichia coli, Proteus spp., Pseudomonas spp., and Clostridium spp.,* volumes of 40 µL, 60 µL, and 80 µL of the pigment suspensions (equivalent to 40 µg, 60 µg, and 80 µg of pigment respectively) were tested. For *Bacillus spp.,* volumes of 20 µL, 40 µL, and 60 µL (equivalent to 20 µg, 40 µg, and 60 µg of pigment respectively) were evaluated, as preliminary screening indicated higher sensitivity of this organism to the pigment extract at lower concentrations.

Müeller Hinton Agar (MHA) plates were prepared and inoculated with each test organism standardized to approximately 1.5 × 10^8 CFU/mL, using a sterile cotton swab by the lawn culture technique. Wells of 6 mm diameter were bored aseptically into the agar using a sterile cork borer and the respective volumes of the pigment suspension were carefully loaded into the designated wells. Ampicillin (20 µL) was used as the positive control and DMSO alone (20 µL) was used as the negative control to confirm the absence of inherent antimicrobial activity from the solvent vehicle.

All plates were allowed to stand at room temperature for 30 minutes to allow pre-diffusion of the pigment into the agar before incubation. Plates inoculated with bacterial organisms were incubated at 37°C for 24 hours.

Following incubation the zones of inhibition were measured in millimetres using a ruler or Vernier calliper and the diameter of the well (6 mm) was included in the measurement. All experiments were performed in triplicate and results expressed as mean ± standard deviation (SD).

Percentage Inhibition Calculation

The percentage inhibition of the red banana peel pigment relative to the standard antibiotic (ampicillin) was calculated for each test organism at each concentration using the following formula as described by Sökmen *et al.,* (2004).

% Inhibition = (Zone of inhibition of pigment sample / Zone of inhibition of ampicillin) × 100

Where the zone of inhibition values used in the calculation represent the mean diameter (mm) of the inhibition zone recorded for each treatment in triplicate, including the 6 mm well diameter. The percentage inhibition values were calculated to provide a quantitative measure of the antimicrobial potency of the red banana peel pigment relative to the standard antibiotic control, allowing objective assessment of the bioactive potential of the pigment extract across the different test organisms and concentration levels. A higher percentage inhibition value indicates greater antimicrobial potency of the extract relative to ampicillin. All percentage inhibition values were calculated from mean zone of inhibition values and expressed as percentage (%).

Statistical Analysis

All antimicrobial experiments were conducted in triplicate and data expressed as mean ± standard deviation (SD). The zone of inhibition data was subjected to one-way Analysis of Variance (ANOVA) to determine the statistical significance of differences among the three pigment concentrations for each test organism. Tukey's Honest Significant Difference (HSD) post-hoc test was applied following ANOVA to identify which specific

concentration pairs differed significantly from each other. A p-value of less than 0.05 was considered statistically significant.

Application of Natural Pigment in Food Products and Stability Evaluation

Preparation of Pigment-Enriched Yogurt

Fresh plain yogurt was prepared/collected and homogenised by gently stirring to achieve a uniform consistency. The extracted pigment was added in the yogurt at a preset concentration of 0.5% (w/w). The pigment was well blended under aseptic conditions to guarantee even distribution. A control was kept in the form of yogurt without the addition of coloring. Samples were put into aseptic containers and stored at two settings i.e. room temperature (25 ± 2°C) and refrigerator (4 ± 1°C). The samples were evaluated for color stability (visual observation/colour measurement- colour, flavour, appearance, general acceptability), pH changes, Microbial quality (Boo *et al.,* 2012).

Colour Stability (Visual Assessment of Product Stability)

The pigment-incorporated yogurt and lemon juice samples were visually examined during storage on Days 0, 3, 5, and 7 under room temperature (25 ± 2°C) and refrigerated (4 ± 1°C) conditions. The samples were observed for changes in colour intensity, appearance, pigment precipitation, phase separation, and overall visual stability. Any visible changes were recorded and compared with the control samples to evaluate pigment stability during storage (Martin *et al.,* 2017).

pH Measurement

The pH of the pigment-incorporated yogurt and lemon juice samples was determined on days 0, 3, 5, and 7 using a calibrated digital pH meter. The pH meter was calibrated using standard buffer solutions (pH 4.0 and 7.0) before analysis. Approximately 20 mL of each sample was taken, and the electrode was immersed in the sample until a stable reading was obtained. The measurements were recorded in triplicate, and the mean pH value was calculated (Martin *et al.,* 2017).

Viability of Probiotic Bacteria (*Lactobacillus spp*.)

The viability of probiotic bacteria in pigment-incorporated yogurt was evaluated during storage on Days 0, 3, 5, and 7 under refrigerated (4 ± 1°C) and room temperature (25 ± 2°C) conditions. Approximately 1 g of yogurt was aseptically transferred into 9 mL of sterile physiological saline (0.85% NaCl) and homogenized. Serial ten-fold dilutions were prepared up to an appropriate dilution ($10^{-}1$ or $10^{-}2$). Aliquots (0.1 mL) of suitable dilutions were spread onto de Man, Rogosa and Sharpe (MRS) agar plates. The plates were incubated at 37°C for 48 h, after which the colonies were counted. The viable probiotic population was expressed as colony-forming units per gram (CFU/g) of yogurt. The viable counts of the pigment-incorporated yogurt were compared with those of the control yogurt throughout storage to determine whether pigment incorporation affected the survival of probiotic bacteria.

Results and Discussion

Red banana peel was extracted with 70% acidified ethanol to obtain dark reddish-maroon pigment extract. The extraction yields of fresh peel material after filtration, rotary evaporation and freeze drying were also calculated for Batch 1 (10 g) and Batch 2 (20 g) (Fig. 1 and 2). Extraction yield (%) was calculated as weight of dried pigment extract obtained to weight of fresh peel material used expressed in percentage. This is the standard way to calculate the yield of plant extract (Kumar *et al.,* 2024). Extraction Yield (%) = (Weight of dried pigment powder) / (Weight of fresh peel) × 100 The freeze-dried pigment powder was a fine hygroscopic dark red/maroon solid, consistent with the visual properties of anthocyanin-rich plant extracts reported elsewhere (Rosalina *et al.,* 2022). Comparison of two extraction batches (Batch 1 10 g in 50 ml; Batch 2 20 g) gave an extraction efficiency of 10 % indicating that the solid to solvent ratio had little effect on the yield in the range tested. Both extraction batches were prepared with a solid solvent ratio of 1:5 (w/v) and a qualitative visual assessment of the extract from the 20 g batch showed darker pigmentation (Deng *et al.,* 2024) 2022. The observed variation in pigment intensity may be due to natural variation in the amount of anthocyanin in the peel starting material, which is highly affected by the ripeness of fruit and post-harvest storage time (Fu *et al.,* 2018).

FTIR Spectral Analysis

FTIR spectrum of the red banana peel pigment extract showed several characteristic absorption bands for an anthocyanin/flavonoid-glycoside structure. A broad

intense band at 3281.01 cm −1 was indicative of O–H stretching vibrations Fig 3. The extensive hydroxylation was due to the presence of polyphenolic hydroxyl groups, which are characteristic of anthocyanins and related flavonoids.

The flavylium chromophore, the conjugated structural feature responsible for the characteristic red-purple colouration of anthocyanin pigments, was associated with bands at 1715.30cm-1 and 1602.37cm-1 corresponding to C=O and aromatic C=C stretching vibrations respectively. A strong, sharp band of absorption at 1029.31 cm -1, attributed to C-O-C stretching, confirmed the presence of a glycosidic linkage, and indicated that the extracted pigment was mainly in the glycosylated form (anthocyanin glycoside) instead of the free aglycone Table 1, Fig 4. This was further supported by the presence of aromatic C-H out-of-plane bending vibrations in the region of 700-920 cm -1, consistent with the substituted aromatic ring system characteristic of the anthocyanidin B-ring.

The spectral profile obtained in the present study is in good agreement to FTIR characteristics previously reported for anthocyanin extracts from banana. Senevirathna *et al.,* (2024) observed the comparable diagnostic bands for cyanidin standards and banana inflorescence anthocyanin extracts consisting of broad O-H stretching band at ~3300 cm-1, aromatic C=C stretching band at ~1600 cm-1 an Similarly, Memon *et al.,* (2008) also reported similar peaks for general banana peel constituents (O–H ~3310 cm^{-1}, C–H ~2920 cm^{-1}, C=O ~1734 cm^{-1}, aromatic C=C ~1613 cm^{-1}, C–O ~1035 cm^{-1}) which further confirmed the present spectral assignments. These results together provide structural support for the anthocyanin/flavonoid glycoside nature of the pigment extracted from red banana peel. As the extract analysed is a crude, unpurified preparation, it is probable that some spectral regions contain overlapping contributions from co-extracted carbohydrates and polysaccharides, such as residual pectin or cellulose fragments from the peel matrix, especially the C-H stretching band at 2927.36 cm-1 and the C-O-C stretching bands. This is a common feature of crude plant extracts and does not affect the overall structural assignment, however further purification (e.g. column chromatography) would be necessary to obtain a spectrum attributable exclusively to the isolated anthocyanin fraction.

The antioxidant potential of the extracted pigment was evaluated using the DPPH free radical scavenging assay, with Vitamin C (ascorbic acid) serving as the standard antioxidant. The relatively high DPPH radical scavenging activity (68%) observed in the extracted pigment may be attributed to the presence of bioactive compounds capable of donating hydrogen atoms or electrons to stabilize free radicals.

Similar antioxidant activities have been reported for microbial pigments such as carotenoids, prodigiosin, violacein, and melanin, which possess strong free radical scavenging properties. The antioxidant potential demonstrated in the present study supports the possible application of the pigment as a natural antioxidant in functional foods, nutraceuticals, and pharmaceutical products.

The extracted pigment exhibited a DPPH radical scavenging activity of 68.0 ± 0.58%, whereas the standard Vitamin C showed a significantly higher scavenging activity of 95.0 ± 0.58% Table 2 Figure 5.

Although the antioxidant activity of the extracted pigment was lower than that of the standard, it demonstrated substantial free radical scavenging ability, corresponding to approximately 72% of the activity exhibited by Vitamin C. The observed antioxidant activity indicates that the pigment possesses appreciable hydrogen- or electron-donating capacity, enabling it to neutralize DPPH free radicals effectively.

These findings suggest that the extracted pigment is a promising natural source of antioxidants and may have potential applications as a bioactive ingredient in functional foods, nutraceuticals, and pharmaceutical formulations (Sujithra and T.Manikkanda 2019).

Table.1 FTIR analysis of the extracted Pigment

Wavenumber (cm^{-1})	Functional Group / Vibration Structural Significance	Structural Significance
3281.01	O–H stretching (broad, H-bonded)	Stretching (wide, hydrogen-bonded) Phenolic/alcoholic hydroxyl groups – commonly found on anthocyanins, flavonoids and sugar moieties. The breadth and intensity of this band is typical of polyphenol-rich plant extracts.
2927.36	C–H stretching (asymmetric, aliphatic CH_2/CH_3)	Aliphatic side chains — from sugar backbone (glycoside) and residual plant lipids/waxes.
1715.30	C=O stretching	Carbonyl group -carboxylic acid or ester; consistent with organic acids (e.g., citric acid residue from your acidified extraction) or acylated anthocyanin derivatives.
1602.37	Aromatic C=C stretching (ring skeletal vibration)	Aromatic ring of the flavylium/anthocyanidin core — a key diagnostic band for flavonoid-class pigments.
1396.96	O–H bending / symmetric COO^- stretch	Phenolic O–H bending or carboxylate group, often overlapping in plant polyphenol spectra.
1243.99	C–O stretching (phenolic/ester)	Phenolic C–O or ester linkage — again consistent with flavonoid substitution pattern.
1029.31	C–O–C stretching (glycosidic linkage)	Most diagnostically important peak — this strong, sharp band is the classic signature of the sugar (glycoside) moiety attached to the anthocyanidin aglycone (e.g., glucose, rutinoside).
921.09 / 866.84	C–H out-of-plane bending (anomeric region)	Consistent with the anomeric carbon region of the attached sugar unit.

Table.2 DPPH Free radical scavenging Activity

Sample	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean ± SD (%)
Extracted pigment	68	69	67	68.00 ± 1.00^b
Vitamin C (Standard)	95	96	94	95.00 ± 1.00^a

Values are expressed as mean ± standard deviation (n = 3). Statistical significance between the extracted pigment and the standard (Vitamin C) was determined using an unpaired Student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

Table.3 Antimicrobial study of the extracted pigment

Zone of inhibition in mm							
S. No.	**Pigment (µl)**	***Bacillus spp.***	***Pigment (µl)***	***E.coli***	***Proteus spp.***	***Pseudomonas spp.***	***Clostridium spp.***
1.	20	15	40	31	11	13	18
2.	40	16	60	35	13	15	20
3.	60	19	80	40	16	19	28
Antibiotic	20	34	20	19	32	30	19
DMSO	20	0	0	0	0	0	0

Table.4 Antimicrobial Activity of Pigment Extract Expressed as Zone of Inhibition (mm): Mean ± Standard Deviation

S. No.	Pigment (µL)	*Bacillus* spp. (mm)	Pigment (µL)	*E. coli* (mm)	*Proteus* spp. (mm)	*Pseudomonas* spp. (mm)	*Clostridium* spp. (mm)
1	20	15.33 ± 0.58^{c}	40	31.33 ± 0.58^{c}	11.33 ± 0.58^{c}	13.33 ± 0.58^{c}	18.33 ± 0.58^{c}
2	40	16.33 ± 0.58^{b}	60	35.33 ± 0.58^{b}	13.33 ± 0.58^{b}	15.33 ± 0.58^{b}	20.33 ± 0.58^{b}
3	60	19.33 ± 0.58^{a}	80	40.33 ± 0.58^{a}	16.33 ± 0.58^{a}	19.33 ± 0.58^{a}	28.33 ± 0.58^{a}
Antibiotic	20	34.33 ± 0.58^{A}	20	19.33 ± 0.58^{B}	32.33 ± 0.58^{A}	30.33 ± 0.58^{A}	19.33 ± 0.58^{B}
DMSO	20	0.00 ± 0.00^{D}	20	0.00 ± 0.00^{c}	0.00 ± 0.00^{D}	0.00 ± 0.00^{D}	0.00 ± 0.00^{D}

Values are expressed as mean ± standard deviation (n = 3). Different lowercase superscript letters within each microorganism indicate significant differences among pigment concentrations according to one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$). Antibiotic served as the positive control and DMSO as the negative control.

Table.5 pH of Pigment-Enriched Yogurt during Storage

Day	Refrigeration Control	Refrigeration Sample	Room Temperature Control	Room Temperature Sample
0	4.60	4.62	4.60	4.62
3	4.55	4.58	4.48	4.52
5	4.48	4.52	4.35	4.40
7	4.42	4.46	4.20	4.28

Table.6 pH of Pigment-Enriched Lemon Juice during Storage

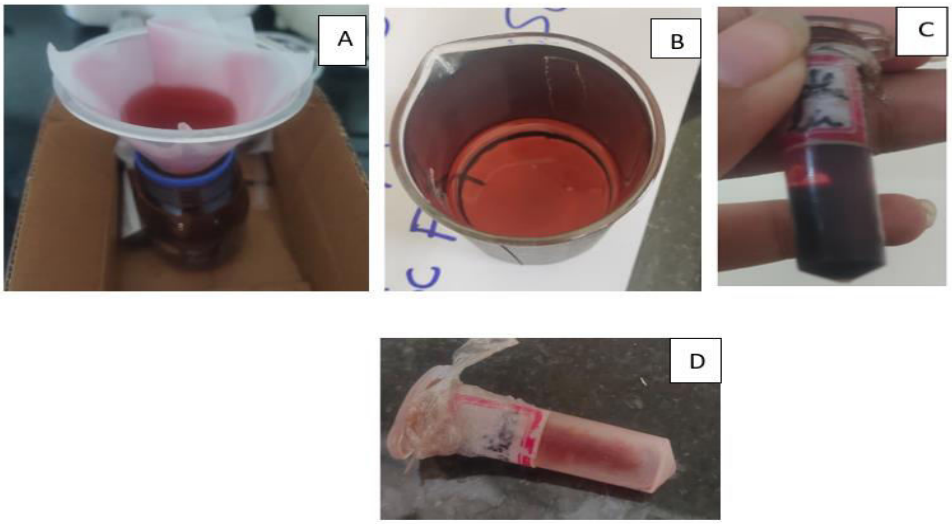
Day	Refrigeration Control	Refrigeration Sample	Room Temperature Control	Room Temperature Sample
0	2.65	2.68	2.65	2.68
3	2.62	2.65	2.55	2.60
5	2.58	2.61	2.45	2.50
7	2.54	2.58	2.35	2.40

Table.7 Visual examination of *lactobacilli* on MRS media plates

Sample	Observation
Control yogurt	TNTC (Too Numerous to Count); abundant Lactobacillus growth (both refrigeration and room temperature maintained)
Pigment-incorporated yogurt	TNTC (Too Numerous to Count); abundant Lactobacillus growth (both refrigeration and room temperature maintained)

Figure 1 A,B,C Collection of Red banana peel for the extraction of the Pigment

Figure.2 Extraction of the pigment A&B filtration, C-sample after rotary evaporation, D-sample after Lyophilization



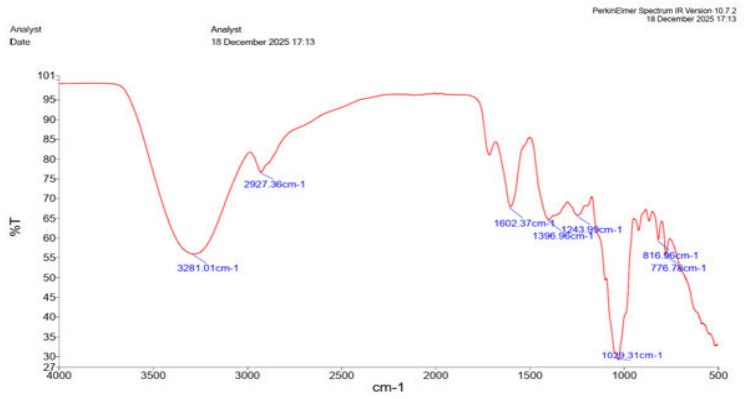


Figure 3 FTRI Spectra results (%T Vs Wave number)

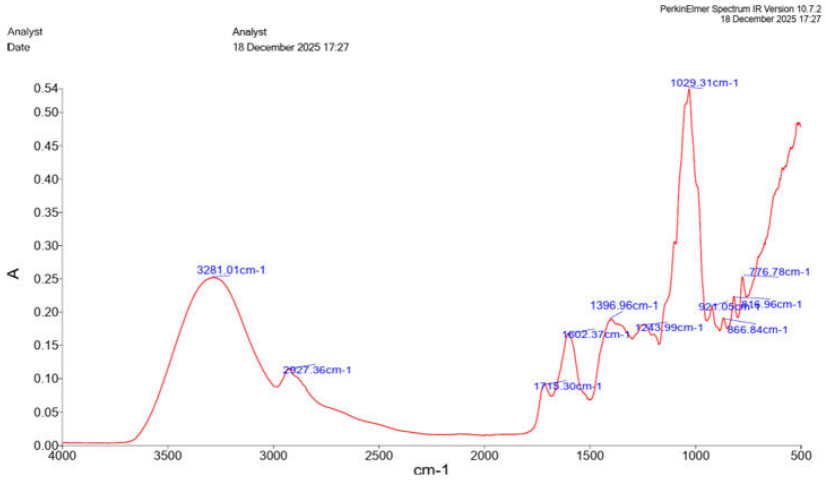


Figure 4 FTRI Spectra results (Abs Vs Wave number)

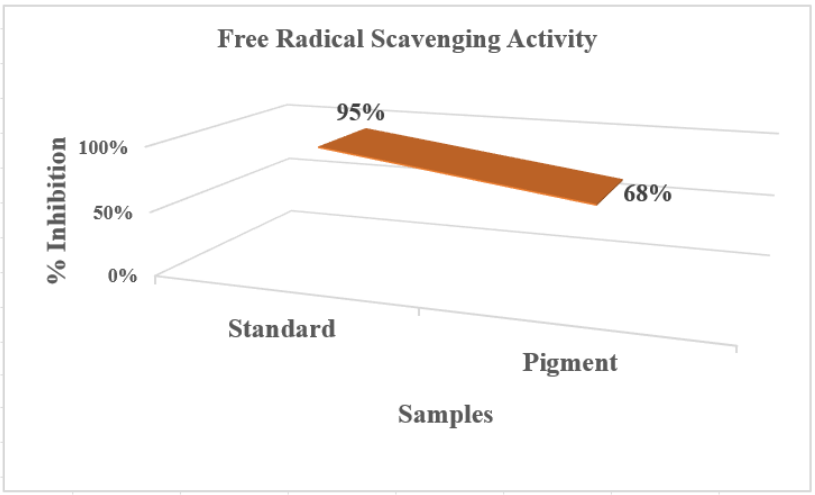


Figure 5 Free Radical Scavenging Activity

Figure 6 Incubation of Clostridium under Anaerobic conditions

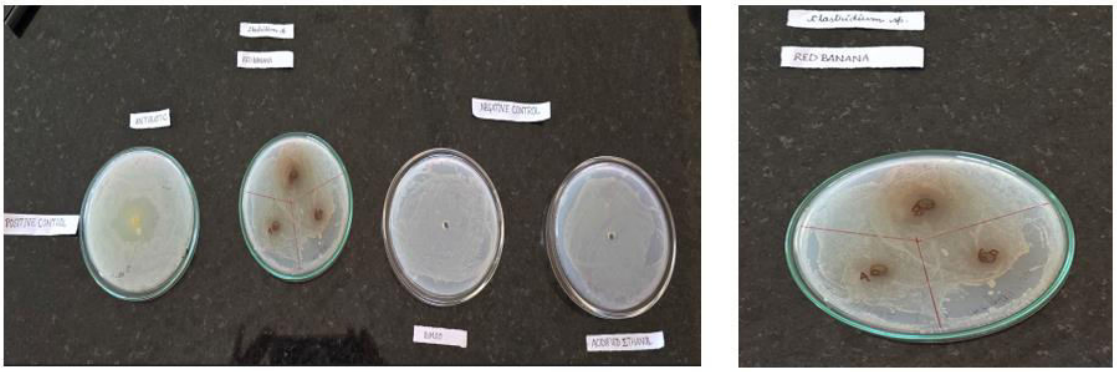


Figure 7 Antimicrobial activity of the pigment against *Clostridium spp.*

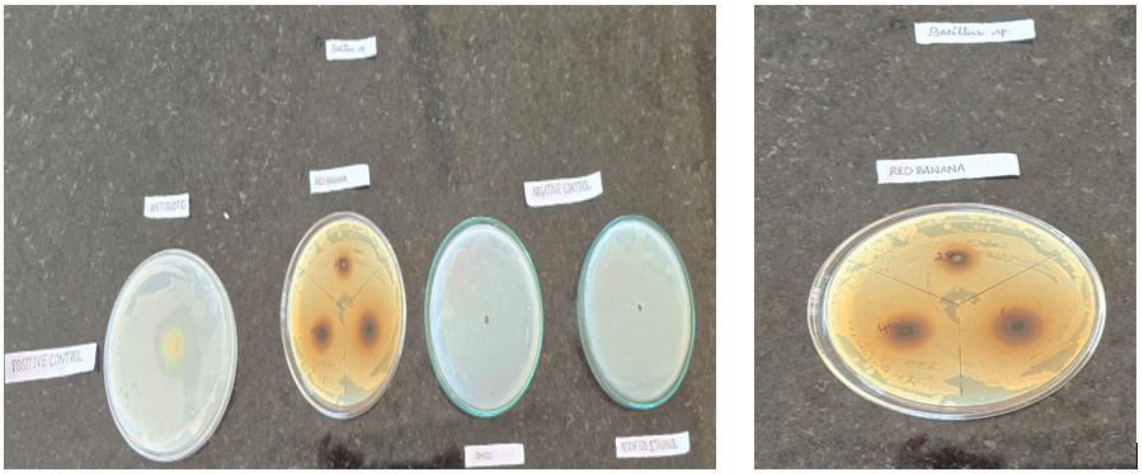


Figure 8 Antimicrobial activity of the pigment against *Bacillus spp.*

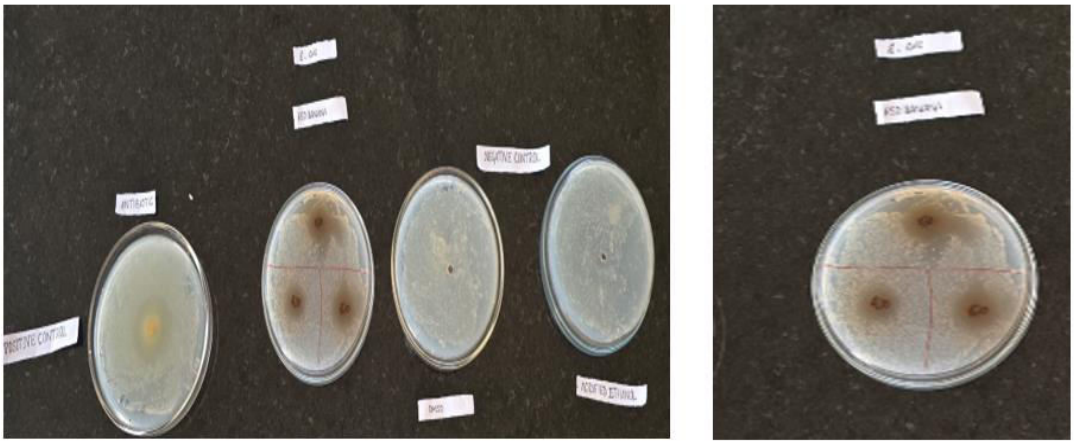


Figure 9 Antimicrobial activity of the pigment against *E.coli*

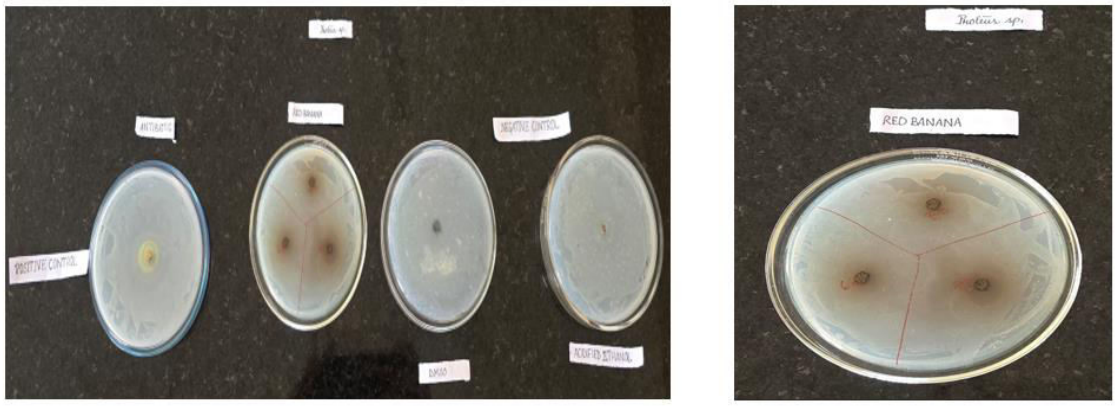


Figure 10 Antimicrobial activity of the pigment against *Proteus spp.*

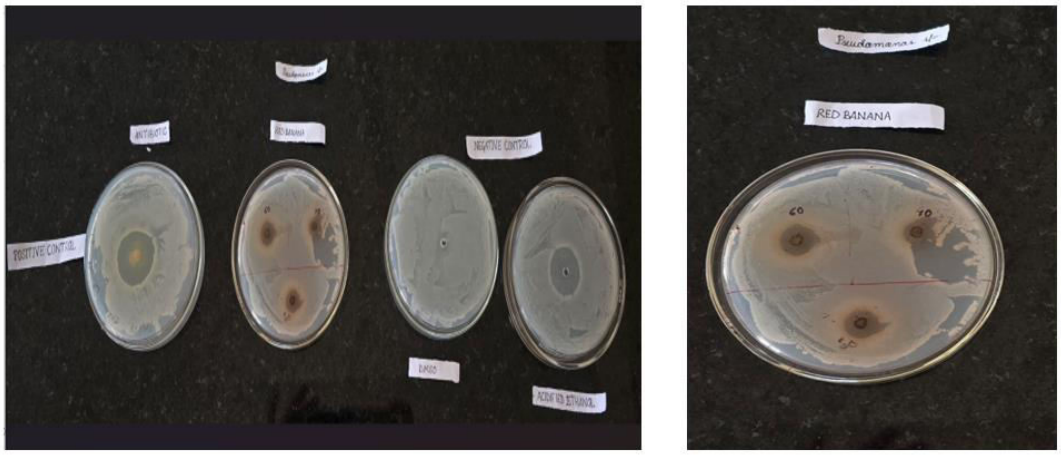
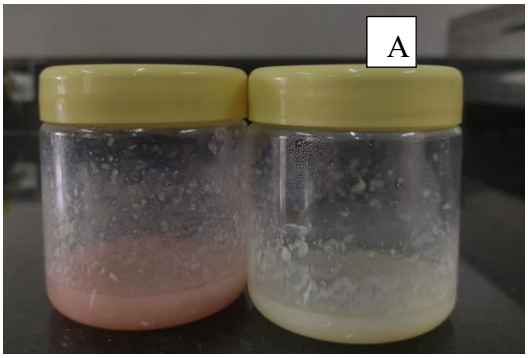


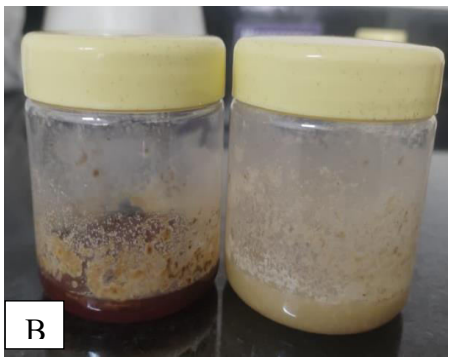
Figure 11 Antimicrobial activity of the pigment against *Pseudomonas spp.*

Figure.12 A-Yogurt sample Control, B-Yogurt sample with pigment on 3rd day (Refrigerated)

Figure.13 A-Yogurt sample, B- Yogurt sample Control with pigment on 7th day (Refrigerated)

Figure.14 Juice samples A-sample with pigment and Control without pigment on 3rd day (Refrigerated), B-A-sample with pigment and Control without pigment on 7th day (Refrigerated).





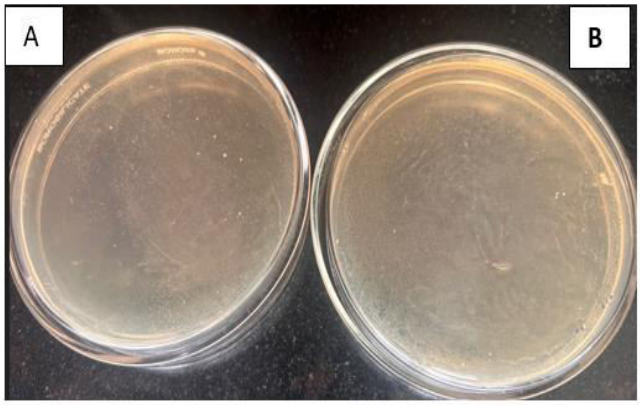


Figure 15 A- *Lactobacilli* on the 7th day of refrigerated sample(Yogurt) B-*Lactobacilli* on the 7th day of refrigerated sample (Room Temperature)

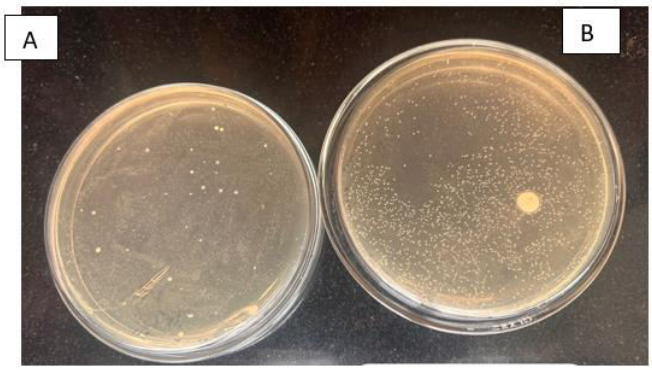


Figure 16 A- *Lactobacilli* on the 7th day of Refrigerator temperature sample , B- *Lactobacilli* on the 7th day of room temperature sample

Antimicrobial studies

The antimicrobial activity of the acidified ethanol extract of red banana (*Musa acuminata*) peel pigment was evaluated against five pathogenic microorganisms, *Bacillus spp., Escherichia coli, Proteus spp., Pseudomonas spp., and Clostridium spp*. Fig 6,7,8,9,10,11 using the agar well diffusion method at three concentrations (20, 40, and 60 µl). Ampicillin (20 µ) served as the positive control while acidified ethanol (20 µl) and DMSO (20 µ) served as negative controls Table 3. The results are presented in Table 4 as mean ± SD (n=3).

The red banana peel pigment extract exhibited clear concentration-dependent antimicrobial activity against all five test organisms, with zones of inhibition increasing progressively from 20 µl to 40 µl to 60 µl concentrations. At the lowest concentration (20 µl), inhibition zones ranged from 11.00 ± 1.00 mm (*Proteus spp*.) to 18.00 ± 1.00 mm (*Clostridium spp*.). At 40 µl, zones ranged from 13.00 ± 1.00 mm (*Proteus spp*.) to 20.00 ± 1.00 mm (*Clostridium spp*.). At the highest concentration (60 µl), zones ranged from 16.00 ± 1.00 mm (Proteus spp.) to 28.00 ± 1.00 mm (*Clostridium spp*.).

Among the five test organisms, *Clostridium spp*. was the most sensitive, recording the highest zone of inhibition of 28.00 ± 1.00 mm at 60 µl, which represented 93.3% of the ampicillin control activity (30.00 ± 1.00 mm). *Proteus spp*. was the least sensitive organism at 20 and 40 µl; however at 60 µl (16.00 ± 1.00 mm) it achieved 84.2% of the ampicillin activity (19.00 ± 1.00 mm), indicating significant dose-dependent potentiation. One-way ANOVA revealed that differences among the three pigment concentrations were statistically significant ($p < 0.05$) for all five test organisms. For *Clostridium spp*. the calculated F value was 84.00, far exceeding the critical value of 5.14 (df 2, 6; $p = 0.05$). Tukey's HSD post-hoc test (HSD = 2.51 mm) revealed that the 60 µl concentration was significantly superior to both 20 µl and 40 µl concentrations ($p < 0.05$), while 20 µl and 40 µl did not differ significantly from each other for most organisms.

Both negative controls — acidified ethanol and DMSO — showed no inhibitory activity (0.00 ± 0.00 mm) against most organisms, with only marginal activity of acidified ethanol against *Proteus spp.* (3.67 ± 0.58 mm) and *Clostridium spp.* (1.67 ± 0.58 mm) which was attributed to the inherent mild antimicrobial property of ethanol at the tested volume and considered negligible in the context of overall antimicrobial evaluation (Sweidan *et al.,* 2023 & Hussain *et al.,* 2022).

The pH of both yogurt and lemon juice decreased gradually during storage under both refrigerated and room-temperature conditions Table 5 & 6. The reduction in pH was more pronounced in samples stored at room temperature than under refrigeration. Pigment-enriched samples exhibited slightly higher pH values than their respective controls throughout storage, indicating that the incorporated pigment had minimal influence on product acidity while remaining stable. Refrigerated storage effectively minimized pH changes, suggesting

improved preservation of product quality Fig 12, 13 &14 (Furtado *et al.,* 1993).
Visual examination of the MRS agar plates revealed dense growth of Lactobacillus colonies in both control and pigment-treated yogurt samples, indicating high probiotic viability.

No noticeable reduction in colony density was observed in the pigment-containing samples, confirming that the natural pigment had no detrimental effect on probiotic survival Table 7, Fig 16,17 (Yosuf *et al.,* 2016).

In conclusion, the present study has successfully demonstrated the extraction, characterization and application of natural anthocyanin rich pigment from red banana (*Musa acuminata*) peel. The acidified ethanolic extract was lyophilized and gave a stable dry pigment powder. FTIR spectrum of the pigment showed characteristic absorption peaks for O—H, C=O, C=C and C—O—C groups, which suggested the pigment to be a compound of anthocyanin class. The antimicrobial activity of the extracted pigment was found to be significantly concentration dependent against all the five pathogenic organisms tested, *Bacillus spp., E. coli, Proteus spp., Pseudomonas spp*. and *Clostridium spp*. (one-way ANOVA and Tukey's post hoc test, $p < 0.05$). The application in juice and yogurt food model systems confirmed successful impartation of red-purple coloration with acceptable stability under refrigerated and low-light storage conditions. Together, these results suggest that red banana peel is a sustainable source of a natural multifunctional pigment with a dual colorant and antimicrobial function and its great potential in clean-label food product development through the valorization of agricultural by-product waste.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this study. The research was conducted purely for academic and scientific purposes. No funding was received from any commercial organization.

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Author Contributions

Sujatha Bachu: Conceived and designed the study; developed the methodology; supervised the research; validated the experimental work; interpreted the results; reviewed and edited the manuscript; and approved the final version. Abhirupa Kundu: Performed pigment extraction, antimicrobial experiments, data collection, analysis, and drafted the manuscript. Bijjam Tulsi: Conducted laboratory experiments, probiotic viability studies, data curation, and assisted in manuscript preparation. Deeksha Suresh Naik: Performed yogurt and fruit juice application studies, sensory evaluation, and data analysis. Yuktha Suresh Naik: Conducted FTIR characterization, statistical analysis, prepared figures and tables, and assisted with manuscript editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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