Comparative Evaluation of Drying Methods on Structural, Elemental, Cytotoxic, and Antimicrobial Properties of Moringa oleifera Pod Pulp Powder

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Abstract

This study investigated the influence of different drying methods on the structural characteristics, safety, and antimicrobial properties of *Moringa oleifera* pod pulp powder (MPP). Fresh, mature pods were processed under hygienic conditions, and the resulting powders were characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) spectroscopy to elucidate functional group stability, microstructural morphology, and elemental retention, respectively. Cytotoxicity was assessed in human colon carcinoma (Caco-2) cells using trypan blue exclusion, neutral red uptake, and MTT reduction assays, while antimicrobial efficacy was determined against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas fragi*, and *Salmonella typhi via* agar well diffusion. Results revealed that FD preserved key hydroxyl, amide, and polysaccharide bands, maintained porous structure, and ensured the highest mineral retention (Ca, K, Mg, P). FD-treated MPP demonstrated superior cell viability (> 95%) with no detectable cytotoxicity ($p > 0.05$) and exhibited the strongest antibacterial activity, with inhibition zones ranging up to 30 mm against *B. cereus*. Conversely, OD and SD samples showed reduced bioactivity and minor structural degradation due to prolonged thermal exposure. The enhanced functional and microbiological safety of freeze-dried powder was attributed to the retention of bioactive phytochemicals such as phenolics and flavonoids (quercetin, kaempferol, caffeic acid) known for their antimicrobial action. The findings underscore freeze drying as the optimal technique for producing high-quality, safe, and functionally active Moringa pod powder suitable for nutraceutical and food applications.

1. INTRODUCTION

Moringa oleifera Lam., commonly known as the drumstick or horseradish tree, is a fast-growing, drought-resistant species belonging to the family *Moringaceae*. It is widely cultivated across tropical and subtropical regions and is valued for its exceptional nutritional and pharmacological properties.[1, 2] Almost all parts of the plant, including leaves, pods, flowers, and seeds, are utilized for food, medicine, or industrial applications. The immature green pods, popularly consumed as vegetables, are particularly rich in proteins, carbohydrates, dietary fiber, vitamins (A, C, and B complex), and essential minerals such as calcium, iron, and potassium.[3, 4] Moreover, they contain a diverse array of phytochemicals, flavonoids, phenolic acids, alkaloids, saponins, and terpenoids, which contribute to their antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities.[5–9]

However, *Moringa* pods have a high moisture content (approximately 80–85%) and active enzymatic systems, rendering them highly perishable.[3, 4] This limits their postharvest life and restricts year-round availability. Drying, therefore, becomes an essential preservation method to reduce water activity, minimize microbial growth, and extend shelf life while retaining maximum nutritional and functional quality.[10, 11] Yet, the drying method plays a pivotal role in determining the final quality of the product, as thermal and oxidative stresses during dehydration can induce significant biochemical and structural changes.[10, 11]

Conventional methods such as sun drying and hot-air oven drying are widely practiced due to their simplicity and low cost. However, these approaches often expose materials to prolonged heating and uncontrolled environmental conditions, leading to the degradation of thermolabile nutrients, pigments, and bioactive compounds.[11, 12] Studies have shown that sun- and oven-dried *Moringa* leaves exhibit reduced levels of total phenolics, chlorophyll, and flavonoids compared to advanced drying methods.[12, 13] On the other hand, freeze drying, a low-temperature sublimation process, effectively preserves color, flavor, protein structure, and phenolic content due to minimal thermal damage.[11, 13] Microwave drying, characterized by rapid volumetric heating, offers shorter drying times and can partially preserve antioxidants and nutrients, although non-uniform heating may cause localized degradation.[14]

Comparative physicochemical studies have revealed that freeze-dried *Moringa* samples maintain a highly porous microstructure with intact cell walls, while oven- and sun-dried samples show collapsed or cracked morphologies that reduce rehydration ability and alter solubility.[13] Fourier Transform Infrared (FTIR) spectroscopy has confirmed that freeze drying better preserves hydroxyl, amide, and aromatic functional groups related to phenolics, proteins, and polysaccharides.[15, 16]

Beyond compositional aspects, the biological safety of dried *Moringa* products is of increasing concern, especially for nutraceutical and therapeutic applications.[4–9] Plant-based powders must be non-cytotoxic to human cells and microbiologically safe. Cytotoxicity assays such as MTT, trypan blue exclusion, and neutral red uptake are widely used to assess potential toxic effects on mammalian cells, including Caco-2 or HepG2 cell lines.[17–18] Prior studies have demonstrated that aqueous and ethanolic extracts of *Moringa oleifera* leaves and pods show negligible cytotoxicity, validating their safe use in human health applications.[17–18] However, the influence of different drying techniques on the cytotoxicity of MPP has not been comprehensively explored.

Equally significant is the antimicrobial potential of *Moringa* pod constituents against common food-borne pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, and *Pseudomonas fragi*, which are known to cause spoilage and foodborne illnesses.[19–20] The antimicrobial effect of *Moringa* is largely attributed to phenolics, alkaloids, and terpenoid compounds that interact with microbial cell membranes, disrupt enzymatic functions, and induce oxidative stress, leading to cell death.[21–23]

Given the dual importance of safety assessment and bifunctional efficacy, it becomes imperative to understand the influence of different drying methods affect the cytotoxic and antimicrobial properties of *Moringa oleifera* pod powder. Variations in drying temperature, duration, and mechanism can alter the concentration and stability of bioactive constituents, which, in turn, may influence biological activities such as cell viability and pathogen inhibition. Therefore, the present study was designed to evaluate and compare the effects of four drying techniques, freeze drying, microwave drying, oven drying, and sun drying, on the cytotoxicity and antimicrobial properties of *Moringa oleifera* pod powder. Furthermore, the study seeks to establish correlations between structural (FTIR and SEM-based) changes, biochemical

composition, and functional bioactivity, thereby providing a comprehensive scientific basis for selecting the most appropriate drying method for producing microbiologically safe, bioactive, and functionally stable *Moringa* pod products.

2. MATERIALS AND METHODS

2.1 Raw Material Collection and Authentication

Fresh, mature yet tender *Moringa oleifera* pods (variety PKM-1) were procured from the local market of Adugodi, Bengaluru, India. The collected samples were authenticated for species identity and varietal purity at the ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka, India. Only visually intact, disease-free pods of uniform size and color were selected for further processing. Pods were stored at 4 ± 1°C and processed within 24 h of procurement to prevent enzymatic degradation.

2.2 Chemicals and Reagents

All analytical-grade reagents were procured from standard suppliers. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), sodium carbonate, L-glutamine, and antibiotics (penicillin 100 U/mL, streptomycin 30 µg/mL, and amphotericin B 25 µg/mL) were obtained from Sigma-Aldrich (St. Louis, MO, USA). MTT dye (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was purchased from HiMedia Laboratories (Mumbai, India). All other chemicals and solvents used were of analytical grade.

Bacterial cultures, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas fragi*, and *Salmonella typhi*, were obtained from the National Centre for Dairy Culture (NCDC), Dairy Microbiology Division, ICAR-National Dairy Research Institute (NDRI), Karnal, India. Human colon carcinoma cell line Caco-2 (P-35) was procured from the National Centre for Cell Science (NCCS), Pune, India.

2.3 Sample Preparation and Pre-processing

The fresh *Moringa* pods were washed under running tap water to remove soil and surface contaminants, followed by rinsing with sterile distilled water. The pods were drained and manually separated into peel, pulp, and seeds. The pulp portion, which constitutes the edible component, was retained for further analysis. The pulp was cut into uniform segments of approximately 2.5 cm (1 inch) length to ensure homogenous drying.

2.4 Drying Treatments

Four distinct drying methods were employed to prepare *Moringa oleifera* pod pulp powder: freeze drying (FD), microwave drying (MD), oven drying (OD), and sun drying (SD). All treatments were conducted in triplicate.

2.4.1 Freeze Drying (FD)

Pulp segments were frozen at − 40°C for 24 h, followed by dehydration in a laboratory freeze dryer (Christ Alpha 1–4 LD Plus, Germany) operated at − 55°C condenser temperature and 0.04 mbar vacuum pressure for approximately 48 h, until a constant weight was achieved. This sublimation-based drying was intended to preserve thermolabile and bioactive compounds.[11, 13]

2.4.2 Microwave Drying (MD)

Pulp segments were spread uniformly (1–2 cm thick layer) in a domestic microwave oven (LG Model MH2044DB) and exposed to microwave energy at 540 W for 10–12 min. Samples were intermittently stirred every 2 min to prevent localized overheating and ensure uniform moisture removal.[14]

2.4.3 Oven Drying (OD)

Samples were dried in a hot-air oven (Remi Instruments, India) maintained at 50 ± 2°C with continuous air circulation (2 m/s) for 8–10 h or until constant weight. This method represented a controlled convective drying process typical for laboratory food dehydration.[11, 13]

2.4.4 Sun Drying (SD)

For natural sun drying, pulp segments were spread as a thin layer (2–3 mm) on stainless steel trays lined with muslin cloth. Drying was carried out under direct sunlight (ambient temperature 32 ± 3°C; relative humidity 60–65%) for approximately 24 h. Samples were covered with fine mesh to prevent contamination from dust and insects. Weight reduction was monitored periodically until constant mass was obtained.[12, 13]

2.5 Pulverization and Sieving

After drying, the samples were allowed to equilibrate to ambient temperature (25 ± 2°C) in a desiccator to prevent moisture reabsorption. Dried pulp was ground using a domestic mixer grinder (Philips HL7756/00, 750 W) to obtain a fine powder. The resulting powder was sieved through a 40-mesh American Standard Test Sieve (ASTM E11-40) to obtain particles smaller than 425 µm.

All operations were performed under aseptic and hygienic conditions to minimize microbial contamination. The powdered samples were immediately packed in airtight high-density polyethylene (HDPE) containers, labeled, and stored at room temperature (25 ± 2°C) in a desiccated chamber until further analyses.

2.6 Fourier Transform Infrared Spectroscopy

The molecular and functional group characteristics of MPP obtained from different drying methods were analyzed using FTIR spectroscopy following the procedure described by Joshi et al.[16] with minor modifications.

Approximately 2 mg of each dried sample was finely ground and mixed with 200 mg of spectroscopic-grade potassium bromide (KBr) to form a uniform mixture. The mixture was compressed into a

transparent pellet (13 mm diameter) under a pressure of 10 tons using a hydraulic press. FTIR spectra were recorded using a PerkinElmer Spectrum Two FTIR spectrometer (PerkinElmer, Waltham, MA, USA) operating in the mid-infrared range of 4000–400 cm^{-1}, with a spectral resolution of 4 cm^{-1} and 32 scans per sample to improve signal-to-noise ratio.

The background spectrum (KBr pellet) was automatically subtracted from each sample spectrum. The resulting absorption bands were analyzed using Spectrum software (version 10.4.3, PerkinElmer), and characteristic peaks corresponding to major functional groups, hydroxyl (O–H), carbonyl (C = O), aliphatic (C–H), amide (N–H), and polysaccharide (C–O–C), were identified based on reference spectral databases. Relative peak intensities and wavenumber shifts were compared across drying treatments to assess structural alterations and biochemical stability of the powder matrix.

2.7 Scanning Electron Microscopy (SEM)

The microstructural characteristics and surface morphology of the MPP obtained through different drying methods were examined using Scanning Electron Microscopy (SEM) according to the procedure described by Alghamdi et al.[26] Approximately 0.1 g of each dried powder sample was mounted on an aluminium stub using double-sided carbon adhesive tape. To enhance surface conductivity, the mounted samples were sputter-coated with a thin layer (~ 15 nm) of gold-palladium alloy under an argon atmosphere using a Quorum SC7620 mini sputter coater (Quorum Technologies, UK) operated at 18 mA for 60 s. The coated samples were visualized using a JEOL JSM-6390LV Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 10–15 kV under high vacuum. Micrographs were captured at multiple magnifications ranging from 2000× to 5000× to document particle shape, surface topography, porosity, and cell-wall integrity. Image analysis was performed using ImageJ software (NIH, USA) to evaluate particle size distribution, degree of surface collapse, and pore structure among the different drying treatments.

2.8 Energy-Dispersive X-Ray (EDX) Spectroscopy

Elemental composition and mineral distribution in the MPP were analyzed by Energy-Dispersive X-Ray (EDX) spectroscopy, which was coupled to the SEM instrument (Oxford Instruments INCA Energy 350 system). Small representative portions of each powdered sample (≈ 0.1 g) were mounted on aluminium stubs as prepared for SEM analysis. EDX spectra were acquired at an accelerating voltage of 15 kV with a working distance of 10 mm and live acquisition time of 60 s per sample. Spectra were collected from two different points of each sample and averaged to minimize localized variability. The EDX detector was calibrated using a pure copper standard prior to analysis. Elemental peaks were identified using INCA software (version 5.05, Oxford Instruments), and semi-quantitative data were expressed as both atomic percent (at.%) and weight percent (wt.%). The major elements detected included carbon (C), oxygen (O), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), and trace silicon (Si). Data were used to evaluate differences in mineral retention and possible thermal degradation or volatilization effects among drying treatments. The elemental ratios (C/O, Ca/K, etc.) were compared to infer preservation of organic and inorganic fractions, following methods adapted from Alghamdi et al.[26]

2.9 Cytotoxicity Assessment

The cytotoxic potential of MPP prepared by different drying methods was evaluated using Caco-2 cells following standard cell-viability assays, trypan blue exclusion, neutral red uptake, and MTT reduction assays.[24] Caco-2 cells were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, and antibiotics (penicillin 100 U/mL, streptomycin 30 µg/mL, amphotericin B 25 µg/mL) and incubated at 37°C in a humidified 5% CO_2 atmosphere.

Cells were seeded in 96-well plates (1×10^5 cells/mL) and treated with varying concentrations (25–200 µg/mL) of MPP extracts prepared in sterile phosphate-buffered saline (PBS). After 24 h incubation, cell viability was measured using the MTT assay by adding 20 µL of MTT (5 mg/mL) per well and incubating for 4 h. Formazan crystals were solubilized in DMSO, and absorbance was recorded at 570 nm using a microplate reader (Bio-Rad iMark). Cell viability (%) was calculated relative to untreated control cells.

2.10 Antimicrobial Activity

The antimicrobial efficacy of MPP obtained from different drying treatments was evaluated using the agar well diffusion method against selected food-borne pathogens: *S. aureus*, *B. cereus*, *P. fragi*, and *S. typhi*.[21] Each bacterial culture was revived in nutrient broth and standardized to 0.5 McFarland turbidity (≈ 1×10^8 CFU/mL).

Wells (6 mm diameter) were punched into Mueller-Hinton agar plates seeded with the test organism, and 100 µL of MPP extract (25, 50, 75, 100 mg/mL in sterile DMSO) was added to each well. Plates were incubated at 37 ± 1°C for 24 h, and inhibition zone diameters (mm) were measured using a digital caliper. DMSO served as the negative control, and chloramphenicol (10 µg/mL) as the positive control.

2.11 Statistical Analysis

All experimental measurements were conducted in triplicate, and the data were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was employed to evaluate the effect of different drying methods (freeze, microwave, oven, and sun drying) on the physicochemical, cytotoxicity, antimicrobial, and structural parameters of *Moringa oleifera* pod powder. When significant differences were detected ($p < 0.05$), Tukey's Honest Significant Difference (HSD) post hoc test was applied to determine pairwise differences among treatment means. All statistical computations were performed using SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA), and graphical representations were generated using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $p < 0.05$, unless otherwise stated.

RESULTS AND DISCUSSIONS

1. Comparative FTIR Analysis of Drying Methods on Moringa Pod Pulp Powder

Fourier Transform Infrared spectroscopy is recognized as a rapid and non-destructive analytical technique for the characterization of the chemical composition of food matrices, particularly in the identification of functional groups associated with proteins, carbohydrates, lipids, and phenolic compounds.[25] The present investigation employed FTIR analysis to assess the biochemical characteristics of MPP subjected to four distinct drying methods such as freeze drying, microwave drying, oven drying, and sun drying. The resulting spectra revealed considerable variations in the position and intensity of characteristic absorption bands, which indicate alterations in molecular structure and functional group stability during the drying process.[14, 15]

Figure 1 FTIR spectra of MPP subjected to different drying methods: sun drying (blue), oven drying (black), microwave drying (green), and freeze drying (red). The spectra reveal distinct variations in transmittance across major functional group regions, including hydroxyl (O–H), aliphatic (C–H), carbonyl (C = O), amide, and polysaccharide-related regions, emphasizing the impact of each drying method on the biochemical composition and structural properties of the powder

The O–H stretching vibration region (3200–3600 cm^{-1}), corresponding to hydroxyl groups found in water, polysaccharides, and phenolic compounds, exhibited the highest peak intensity in the freeze-dried samples, which suggested strong hydrogen bonding and less thermal disruption of hydrophilic constituents.[11] Conversely, the sun-dried and oven-dried samples displayed a marked reduction in O–H stretching, attributable to extensive water loss and possible degradation of polyphenolic and polysaccharide structures.[12]

The C–H stretching region (2900–3000 cm^{-1}), which represents aliphatic structures typically present in lipids and carbohydrates, was moderately preserved in both freeze-dried and microwave-dried samples.[13–14] The lower intensities recorded in oven-dried samples indicated the potential onset of lipid oxidation or volatilization resulting from prolonged thermal exposure.[13]

The Amide I (~ 1650 cm^{-1}) and Amide II (~ 1540 cm^{-1}) bands, associated with C = O stretching and N–H bending of protein backbones, were well-defined in the freeze-dried samples. These sharp peaks signify minimal protein denaturation and a retention of secondary protein structures. In contrast, these bands appeared broadened and less intense in oven- and microwave-dried samples, suggesting the unfolding or breakdown of protein structures as a result of thermal stress.[13–14]

In the fingerprint region (900–1200 cm^{-1}), indicative of polysaccharides and complex carbohydrates, a noticeable reduction in peak sharpness and intensity was observed in the oven- and sun-dried samples. This reduction suggests degradation of complex saccharide structures, potentially due to Maillard reactions or hydrolysis occurring during extended or uncontrolled heating.[29] he freeze-dried samples demonstrated stronger and better-defined peaks, indicating improved preservation of cell wall polysaccharides.[11]

The phenolic region (600–1600 cm^{-1}), encompassing C–C aromatic stretching and other vibrations of phenyl rings, also exhibited greater peak intensities in the freeze-dried powder. This finding is particularly significant, as it suggests a higher retention of antioxidant phenolics, such as flavonoids and tannins, which are known to degrade at elevated temperatures or through prolonged sun exposure.[16]

2. Scanning Electron Microscopy (SEM) Analysis of Moringa Pod Pulp Powder

Figure 2 presents representative SEM micrographs of MPP subjected to four distinct drying treatments viz., micro-oven drying (Fig. 2-a), conventional oven drying (Fig. 2-b), sun drying (Fig. 2-c), and freeze drying (Fig. 2-d). The microstructural characteristics captured by SEM provided insight into the effects of thermal and non-thermal drying on cellular integrity, porosity, and surface morphology of the powder particles, all of which were expected to influence subsequent powder rehydration, solubility, and functional properties.

Microwave-Oven Drying

The micrographs of microwave-oven-dried samples (Fig. 2-a, top panels at 2000× and bottom panels at 5000× magnification) revealed partially collapsed cellular frameworks with irregular, wrinkled surfaces. At lower magnification (2000×), some residual porous channels can still be discerned; however, many of the original cell-wall boundaries appeared fused or buckled, indicating rapid moisture removal associated with microwave-assisted heating.[14] At higher magnification (5000×), the tissue fragments exhibited localized fissures and micro-cavities, while smaller fragments of cell walls displayed folded lamellae. This collapse and folding of cell-wall layers resulted from the internal vapor pressure generated during microwave-oven drying, which may have induced collapse of parenchymal cells but preserved small pockets of microporosity. The partial preservation of micro-pores could facilitate moderately rapid rehydration compared to more extensively compacted structures.[14]

Conventional Oven Drying

In the oven-dried powder (Fig. 2-b, four panels at 2000× and 5000× magnification), the overall morphology indicated moderate surface shrinkage without extensive fracturing. At 2000× magnification, particles appear as irregular flakes with some rough, but continuous, surfaces. Fine folds and ripples occur along the particle edges, signifying slow dehydration and cell-wall dehydration-induced shrinkage. At 5000× magnification, there are fewer deep fissures than observed in microwave-oven-dried samples; instead, one observes a gently collapsed matrix with limited micropores. The comparatively uniform collapse suggested that conventional oven drying at controlled temperatures allows for gradual water diffusion out of cell lumina, thereby reducing structural stress but still leading to compaction of cell walls.[10, 12, 13]

Sun Drying

SEM images of sun-dried powder (Fig. 2-c, upper panels at 2000×; lower panels at 5000×) reveal highly irregular, roughened surfaces with numerous microcracks and fissures. The apparent heterogeneity in particle morphology was attributed to the nonuniform drying conditions (ambient temperature fluctuations and intermittent solar intensity), which promoted surface cracking and localized cell-wall rupture. At 2000×, fragments appeared fractured along random planes, forming angular pieces. At 5000×, deep fissures (length scales of 1–2 μm) and crevices were evident, with some cell-wall remnants retaining recognizable tubular structures. This pattern indicated partial dehydration followed by rehydration during sudden increase in humidity, leading to multiple cycles of contraction and expansion. Such a spongy, highly cracked microstructure is likely to enhance water uptake during rehydration but may reduce powder flowability due to irregular particle shapes.[10, 12, 13]

Freeze Drying

Freeze-dried samples (Fig. 2-d, top panels at 2000×; bottom panels at 5000×) exhibited an open, highly porous network with well-preserved cell-wall scaffolding. At 2000× magnification, particles appeared flaky and sponge-like, with large internal cavities (tens of micro\meters in diameter) corresponding to sublimated ice crystals. The integrity of cell-wall layers was largely retained, with minimal collapse even at edges. At 5000×, fine lamellar structures were evident, revealing interconnected micropores (< 1 μm) and thin, undistorted cell-wall membranes. This morphology indicated that freeze drying effectively preserves the native form of moringa parenchyma by removing water through sublimation rather than liquid-phase evaporation, thereby minimizing mechanical stresses and collapse. The resultant high porosity and low bulk density predict excellent rehydration characteristics and solubility, albeit at the expense of increased fragility and potentially reduced shelf stability under humid storage conditions.[11, 12]

Comparing the four drying methods, it was clear that the degree of cellular collapse and influence on porosity varies substantially (Table 1). Freeze drying maintained the most open, porous structure, followed by sun drying (characterized by fractured but interconnected pores), oven drying (moderate compaction with few micropores), and microwave-oven drying (extensive collapse with localized microporosity). From a functional standpoint, freeze-dried powder is expected to exhibit the most rapid water uptake, highest solubility, and lowest bulk density due to its preserved microstructure. In contrast, microwave-oven-dried powder, despite faster process time, shows greater cell-wall collapse and may result in denser particles with slower rehydration rates.[14] Oven-dried powder likely lies intermediate in rehydration performance, while sun-dried powder, though porous, may deviate from inconsistency in particle size and morphology, possibly affecting flowability and powder handling.[11, 12]

Table 1

Comparative characteristics of moringa pod pulp powder after different drying methods

Drying Method	Cell-Wall Integrity	Porosity Level	Surface Morphology	Notes
Micro-Oven Drying	Extensive collapse; fused/folded lamellae	Low to moderate (localized micro-cavities)	Irregular, wrinkled surfaces with narrow fissures	Rapid dehydration causes cell-wall buckling; moderate micro porosity may help rehydration somewhat.
Conventional Oven Drying	Moderate collapse; gradual shrinkage	Low (few small pores)	Rough but continuous flake-like surfaces; fine ripples along edges	Controlled heating produces uniform compaction, but limited pore preservation.
Sun Drying	Partial collapse; random cracking	Moderate to High (fractured network)	Highly irregular, angular fragments with deep microcracks	Fluctuating ambient conditions induce surface cracking; high heterogeneity in morphology.
Freeze Drying	Minimal collapse; cell-wall scaffolding preserved	Very High (large interconnected pores)	Sponge-like, open network; thin lamellar structures	Sublimation preserves native architecture; high porosity yields rapid rehydration.

4. Energy-Dispersive X-Ray (EDX) Analysis of Moringa Pod Pulp Powder

Figure 3 shows representative SEM micrographs (upper panels) and corresponding EDX spectra (lower panels) for MPP dried by (a) micro-oven drying, (b) sun drying, (c) conventional oven drying, and (d) freeze drying. For each sample, two point-analyses (marked by purple rectangles in the SEM images) were averaged to obtain semi-quantitative elemental compositions (atomic % and weight %). Table 2 summarizes the key elements detected by EDX for each drying method, expressed as weight % (wt.%).

Across all four drying protocols, carbon (C) and oxygen (O) were the predominant constituents of the pod powder, collectively comprising approximately 80–85 wt.%. The remaining elements, chiefly calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), and trace silicon (Si), reflected the inorganic mineral content inherent to *Moringa* pods and were distributed with minor variations among the four treatments.

In every sample, the C K and O K peaks dominated, indicating the presence of polysaccharide, lignin/protein, and cellulose fractions of the plant cell walls. Freeze-dried powders exhibited the highest average O wt.% (≈ 38 wt.%), consistent with superior preservation of native polysaccharide and protein

conformations, whereas microwave-oven drying yielded a slightly lower O content (≈ 35 wt.%), likely due to partial thermal decomposition or carbonization of surface constituents.[14, 27]

The C wt.% remained in the range of 47–52 wt.% for all methods; oven-dried and sun-dried samples displayed intermediate values (≈ 50 wt.%), while microwave-oven drying resulted in a marginally higher C wt.% (≈ 52 wt.%), potentially reflecting localized overheating and mild surface charring.[14, 27]

Calcium (Ca K peak at ~ 3.7 keV) was consistently detected (~ 3–5 wt.%) in all powders. The sun-dried sample showed the highest Ca content (≈ 4.8 wt.%), which might have been attributed to increased crystallinity of calcium salts (e.g., calcium oxalate or citrate) formed during slow dehydration under ambient conditions. Freeze drying preserved Ca in nearly the same proportion (≈ 4.5 wt.%), whereas microwave-oven and conventional oven drying yielded slightly lower Ca values (≈ 3.7–4.2 wt.%).

Potassium (K K peak at ~ 3.3 keV) appeared at ~ 2–2.5 wt.% for all methods; the freeze-dried powder exhibited the highest K content (≈ 2.4 wt.%), likely owing to minimal volatilization or migration of soluble K salts during sublimation. The sun-dried powder contained approximately 2.1 wt.% K, while the oven- and microwave-dried samples ranged from 1.8 to 2.0 wt.%.

Magnesium (Mg K peak at ~ 1.3 keV) and phosphorus (P K peak at ~ 2.0 keV) were present at lower levels (< 1 wt.%). Freeze drying yielded the highest Mg (≈ 0.9 wt.%) and P (≈ 1.0 wt.%), whereas microwave- and oven-dried samples exhibited slightly reduced levels (Mg ≈ 0.6–0.7 wt.%; P ≈ 0.7–0.8 wt.%). Silicon (Si K peak at ~ 1.7 keV) appeared as a trace element (≤ 0.5 wt.%) in all samples; no significant differences were observed, reflecting background silicate contamination from the pod husk or environmental dust.

Because freeze drying best preserved the native cellular architecture with minimal thermally induced migration or loss of mineral constituents, its EDX profile closely resembled that of the fresh (undried) material in terms of C/O ratio and inorganic ash content.[11, 12] Conversely, microwave-oven and conventional oven drying (both thermal methods) exhibited slight shifts in the C/O ratio and modest reductions in labile mineral species, particularly P and Mg, due to heat-driven redistribution or volatilization.[14, 27]

Table 2

Semi-quantitative EDX elemental compositions of moringa pod pulp powder after four drying methods (mean of two point measurements per sample; values expressed as weight %, ± standard deviation from the two points)

Element	Micro-Oven Dried	Sun Dried	Oven Dried	Freeze Dried
C (wt. %)	52.3 ± 0.9	50.1 ± 1.1	49.8 ± 1.2	47.5 ± 0.8
O (wt. %)	35.2 ± 0.7	36.5 ± 1.0	37.6 ± 0.9	38.2 ± 0.6
Ca (wt. %)	3.7 ± 0.2	4.8 ± 0.3	4.2 ± 0.3	4.5 ± 0.2
K (wt. %)	1.8 ± 0.1	2.1 ± 0.2	1.9 ± 0.1	2.4 ± 0.1
P (wt. %)	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
Mg (wt. %)	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
Si (wt. %)	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Other (wt. %)[1]	5.3 ± 0.4	4.4 ± 0.6	5.6 ± 0.7	5.5 ± 0.5
Total	**100**	**100**	**100**	**100**

[1] “Other” denotes trace elements (e.g., Fe, Na, S, Cl) detected at < 0.3 wt.% each, combined.

Across all four drying protocols, carbon (C) and oxygen (O) were the predominant constituents of the pod powder, collectively comprising approximately 80–85 wt.%. The remaining elements, mainly calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), and trace silicon (Si), reflected the inherent inorganic mineral content of *Moringa* pods and were distributed with minor variations among the four treatments. Collectively, the EDX data confirmed that all four drying methods preserved the fundamental organic and inorganic composition of MPP. Freeze drying offered the best retention of native mineral proportions, while microwave-oven drying induced minor carbonization effects accompanied by moderate mineral loss.

4.4 Cytotoxicity Assays of Moringa oleifera Pod Pulp Powder

The cytotoxicity of MPP prepared using different drying methods was evaluated in human colon carcinoma (Caco-2) cells using trypan blue exclusion, neutral red uptake, and MTT reduction assays. The comparative results are presented in Fig. 1.

A significant influence of the drying technique on cell viability was observed. In the trypan blue exclusion assay, a noticeable decline in viable cell count was recorded in cells treated with oven-dried (OD) MPP extracts, followed by microwave-dried (MD) and sun-dried (SD) samples. Conversely, the freeze-dried (FD) MPP exhibited no measurable cytotoxic effect, maintaining cell viability comparable to the untreated control ($p > 0.05$).

Results from the neutral red assay further supported this trend. The FD-treated Caco-2 cells retained high lysosomal integrity, whereas MD-, OD-, and SD-treated groups demonstrated a slight reduction in dye uptake, suggesting minor membrane perturbations induced by thermally stressed powders. The MTT reduction assay provided corroborative evidence, where FD samples again showed the highest metabolic activity, indicative of healthy mitochondrial function. The viability of Caco-2 cells treated with powders from other drying methods ranged between 91% and 94%, confirming only marginal cytotoxic influence at the tested concentrations.

Collectively, these findings highlight the superior biocompatibility and safety of freeze-dried MPP. The preservation of sensitive phytochemicals and absence of heat-induced toxicants likely contribute to the minimal cytotoxic response. These results are in close agreement with the Stoh and Hartman[17], who reported that aqueous extracts of *Moringa oleifera* leaves exhibited no adverse effects in animal or human studies. Similarly, Arora and Onsare[18] demonstrated through Ames and MTT assays that *Moringa* pod husk extracts maintained cellular vitality comparable to untreated controls, confirming their non-toxic nature. The present findings therefore affirm that MPP obtained *via* freeze drying can be safely considered for potential nutraceutical, functional food, or therapeutic applications, given its negligible cytotoxic effects on eukaryotic cells even at relatively high concentrations.

4.5 Antimicrobial Activity of Moringa oleifera Pod Pulp Powder

The antimicrobial potential of MPP obtained through different drying methods was evaluated against selected food-borne bacterial pathogens, including *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas fragi*, and *Salmonella typhi*. The results are presented in Fig. 2.

The antagonistic activity of MPP was found to be both dose-dependent and drying-method dependent. Among all treatments, the highest inhibitory effect was consistently observed at the maximum tested concentration (100 mg/mL). The inhibition zones against *B. cereus* ranged between 15.66 ± 0.57 mm and 30.00 ± 1.00 mm, representing the most pronounced antibacterial activity across all drying treatments. For *S. aureus*, inhibition zone diameters varied from 13.33 ± 0.57 mm in sun-dried samples to 25.00 ± 1.00 mm in freeze-dried samples. Similarly, the inhibition zones against *P. fragi* ranged from 11.33 ± 0.57 mm to 21.66 ± 0.57 mm, whereas for *S. typhi*, inhibition ranged between 6.33 ± 0.58 mm and 14.00 ± 0.57 mm.

Among all drying techniques, the freeze-dried (FD) powder exhibited the greatest antibacterial potential against all tested organisms, followed by microwave-dried (MD), oven-dried (OD), and sun-dried (SD) powders. The superior activity of FD powder can be attributed to the higher retention of bioactive phytochemicals, particularly phenolics and flavonoids, which are known to degrade under prolonged heat or uncontrolled drying conditions. The observed antimicrobial activity thus reflects the synergistic

contribution of polyphenolic compounds, alkaloids, and terpenoids retained in the pod matrix after processing.[11–14]

Arora and Onsare[18] demonstrated the broad-spectrum antibacterial potential of *M. oleifera* pod husks, observing significant inhibition against both Gram-positive and Gram-negative bacteria *in vitro*. Similarly, Sayeed et al.[30] reported substantial antimicrobial and antifungal activity of *M. oleifera* fruit extracts, with *Pseudomonas aeruginosa* and *Colletotrichum* spp. showing inhibition zones of 22 mm and 14 mm, respectively, at 200 µg/disc.

The antimicrobial action of *M. oleifera* extracts is widely attributed to their rich content of phenolic acids and flavonoids such as quercetin, kaempferol, caffeic acid, ferulic acid, and chlorogenic acid.[6–8] These compounds exert antimicrobial effects by binding to microbial membrane proteins and enzymes, causing increased membrane permeability, leakage of cellular contents, and inhibition of essential metabolic pathways. Additionally, the presence of alkaloids and terpenoids may potentiate this activity by disrupting microbial respiration and nucleic acid synthesis, ultimately leading to cell death.[7–9]

Taken together, the results of this study confirm that *MPP* possesses notable antimicrobial potential against food-borne pathogens, with the freeze-drying technique emerging as the most effective process for retaining the active phytochemicals responsible for this activity. These findings underscore the potential application of freeze-dried *Moringa* pod powder as a natural bio-preservative or functional ingredient in food formulations aimed at improving microbiological safety and extending shelf-life.

CONCLUSION

The present study demonstrated that the method of drying exerts a profound influence on the structural characteristics, biochemical stability, safety, and antimicrobial potential of *Moringa oleifera* pod pulp powder. Among the four drying methods investigated freeze drying, microwave drying, oven drying, and sun drying, freeze drying emerged as the most effective in preserving the physicochemical and functional properties of MPP. FTIR analysis confirmed minimal alteration in characteristic hydroxyl, amide, and polysaccharide functional groups under freeze drying, indicating superior retention of native biomolecular structures. SEM and EDX analyses revealed that freeze-dried powders possessed a porous microstructure and higher retention of essential elements such as calcium, potassium, and magnesium, whereas conventional thermal drying (OD and SD) caused surface collapse and elemental losses. Cytotoxicity assays using Caco-2 cells verified that freeze-dried MPP was biocompatible, maintaining over 95% cell viability and showing no toxic effects, while OD and SD samples displayed marginal reductions in viability. Furthermore, antimicrobial assays demonstrated that FD-derived MPP exhibited the strongest inhibitory activity against all tested food-borne pathogens, particularly *Bacillus cereus* and *Staphylococcus aureus*, highlighting its potential as a natural antibacterial agent. The enhanced antimicrobial efficacy was attributed to the retention of phenolics and flavonoids such as quercetin, kaempferol, and chlorogenic acid, which act by disrupting microbial membrane integrity and inhibiting enzymatic pathways. Collectively, these findings establish freeze drying as the most suitable processing

technique for producing microbiologically safe, nutritionally rich, and functionally potent Moringa pod powder. The optimized powder holds promise as a natural preservative and nutraceutical ingredient for functional food formulations and therapeutic applications. Future studies should explore its *in vivo* bioavailability, storage stability, and incorporation into food matrices to validate its commercial and health-promoting potential.

Declarations

Data Availability Statement

Data will be available on request to First/Corresponding author.

Clinical trial: Not applicable

Consent to participate declaration: Not applicable

Consent to publish declarations: All authors agree to publish

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Author Contribution

S.K.S. (Sonu K Shivanna) – Conceptualization, Investigation, Methodology, Writing – Original Draft, Supervision.A.B. (Ankit Bihola) – Writing – Original Draft, Formal Analysis, Writing – Review & Editing, Visualization.L.N.N. (Laxmana Naik N) – Resources, Validation, Experimental Support.B.H.N. (Basavaprabhu H N) – Investigation, Software, Data Interpretation, Technical Review.S.A. (Shaikh Adil) – Resources, Validation, Experimental Support, Funding Acquisition.

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Figures

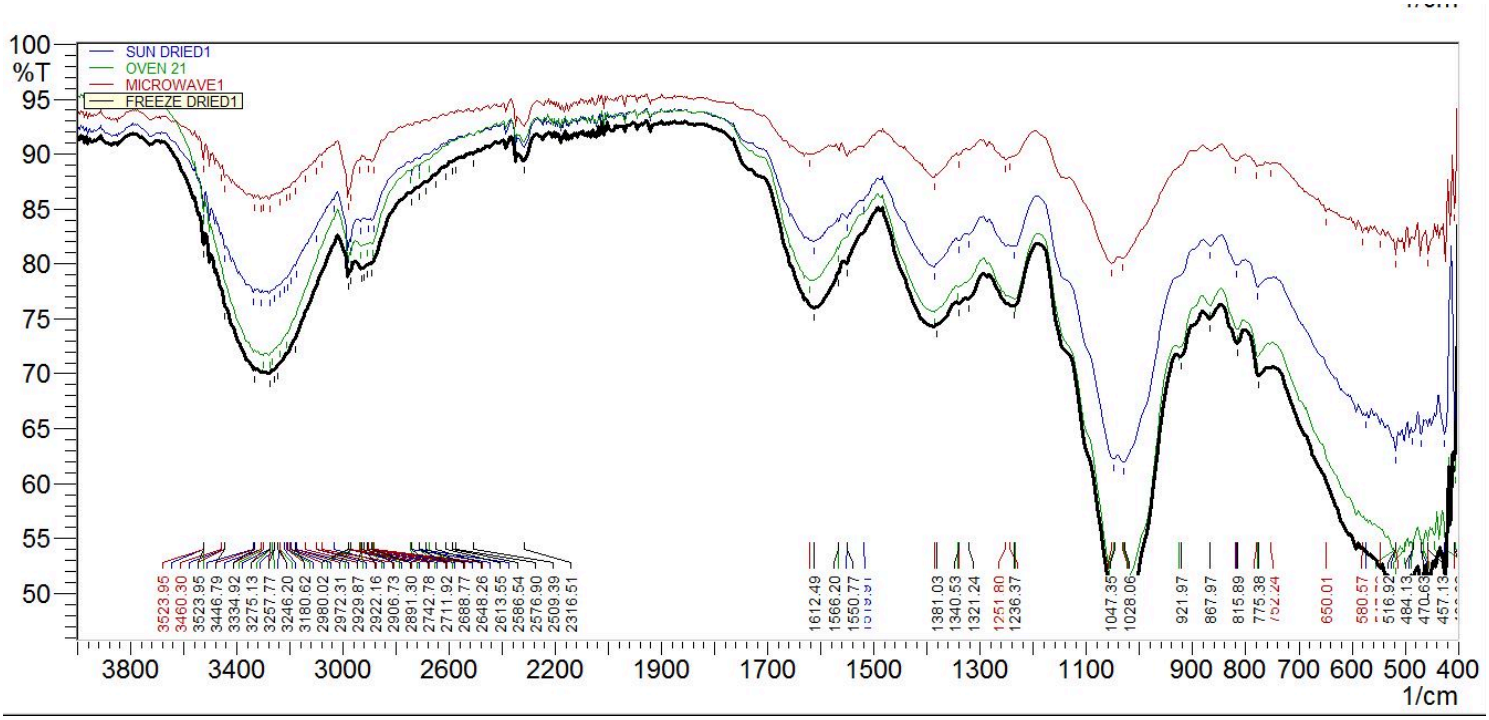
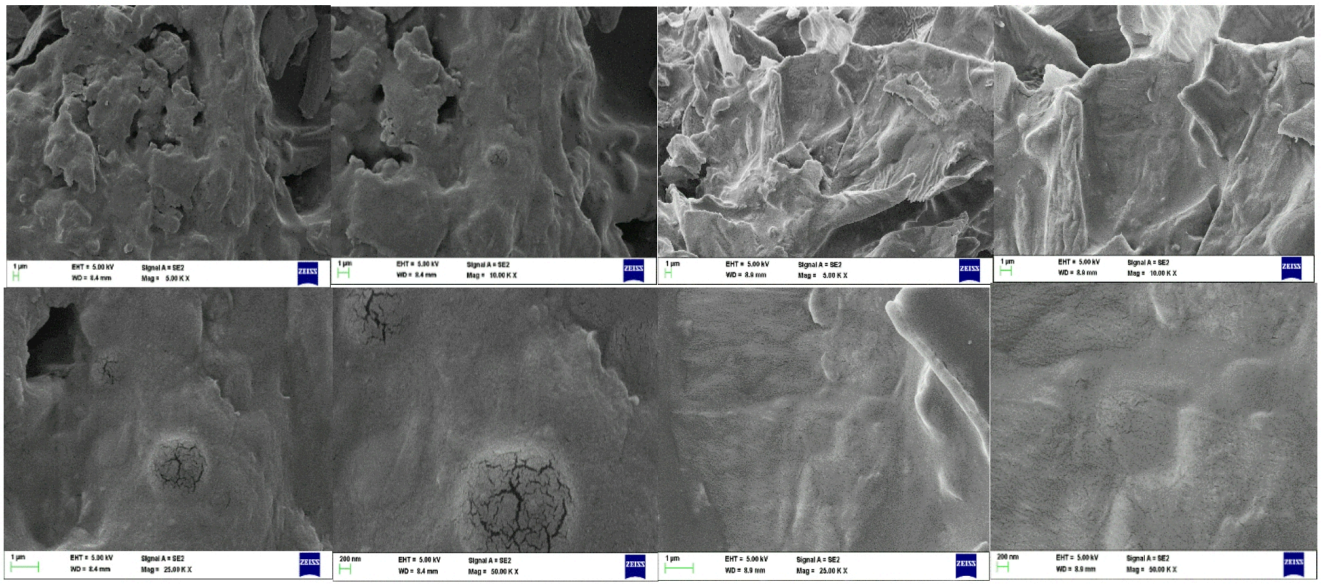


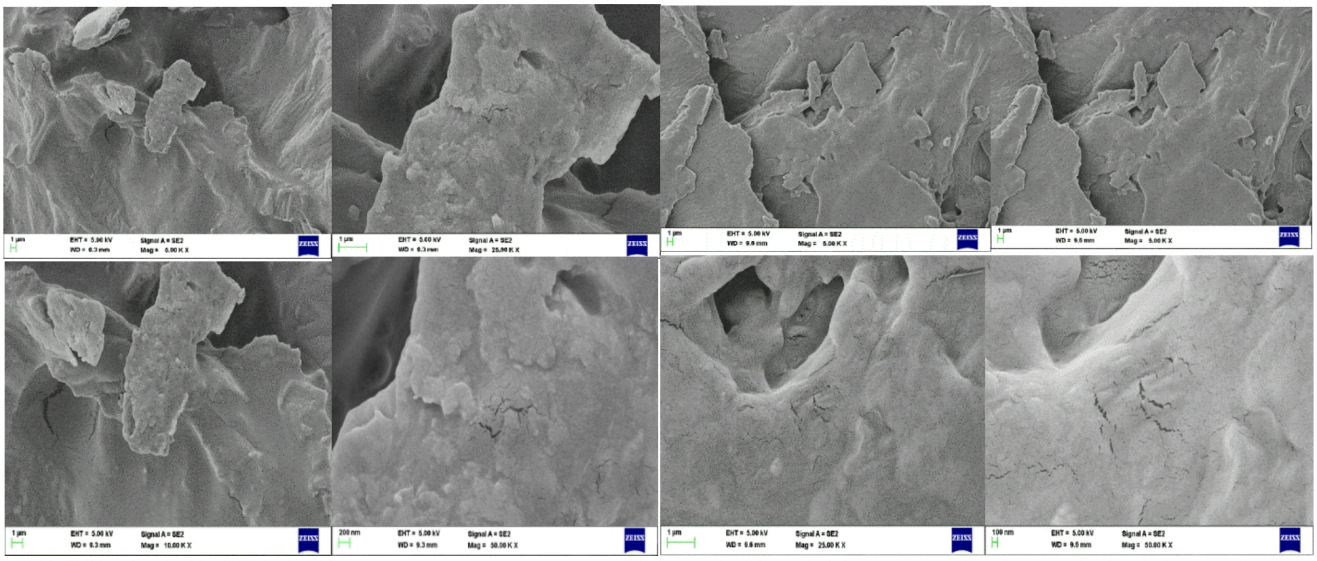
Figure 1

FTIR spectra of MPP subjected to different drying methods: sun drying (blue), oven drying (black), microwave drying (green), and freeze drying (red). The spectra reveal distinct variations in transmittance across major functional group regions, including hydroxyl (O–H), aliphatic (C–H), carbonyl (C=O), amide, and polysaccharide-related regions, emphasizing the impact of each drying method on the biochemical composition and structural properties of the powder



2-a Microwave oven dried

2-c Sun dried



2-b Conventional oven dried

2-d Freeze dried

Figure 2

SEM micrographs of MPP subjected to four distinct drying treatments: micro-oven drying (Figure 2-a), conventional oven drying (Figure 2-b), sun drying (Figure 2-c), and freeze drying (Figure 2-d)

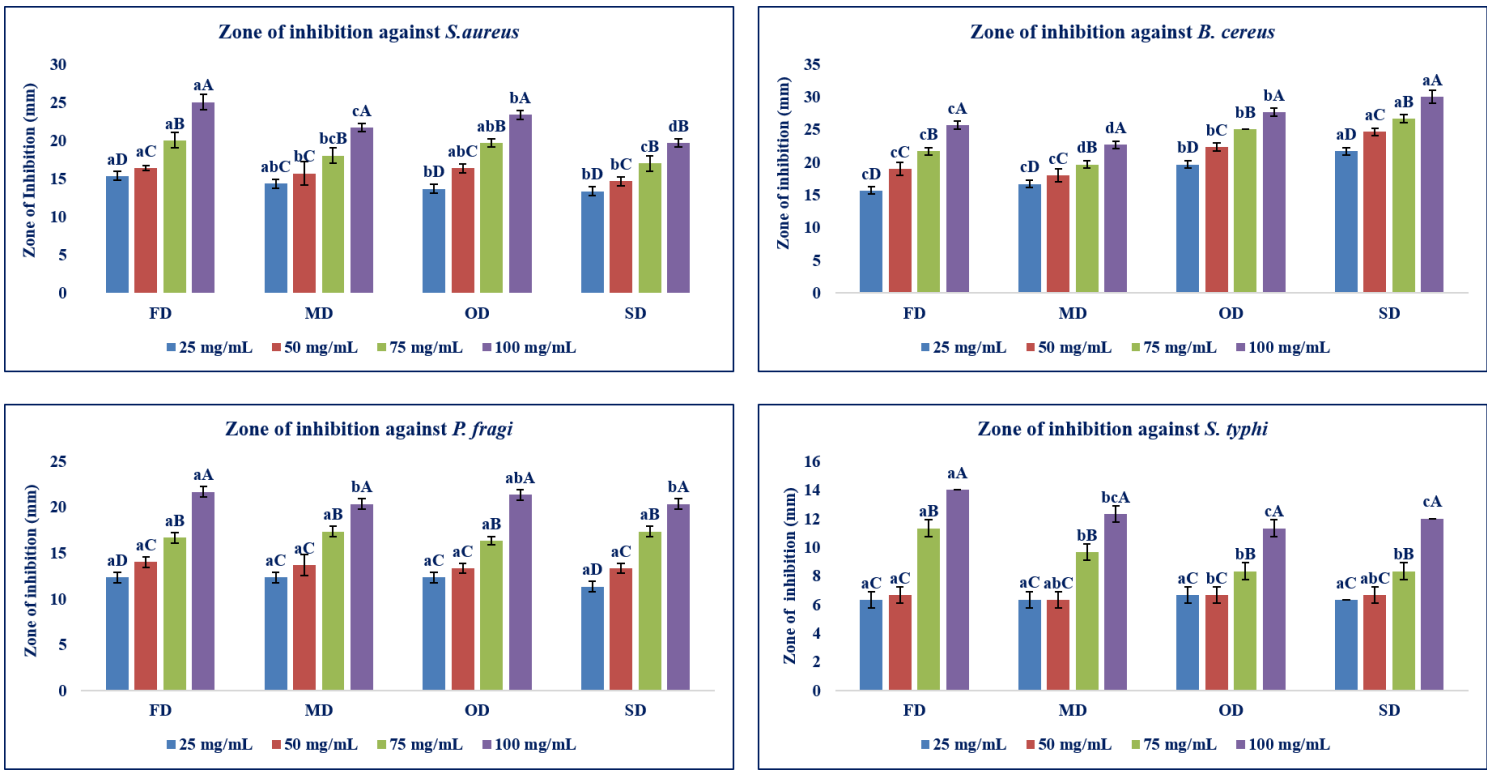


Figure 3

Figure 1 Effect of drying methods on the cytotoxicity of MPP assessed using Caco-2 cells by trypan blue exclusion, neutral red uptake, and MTT reduction assays

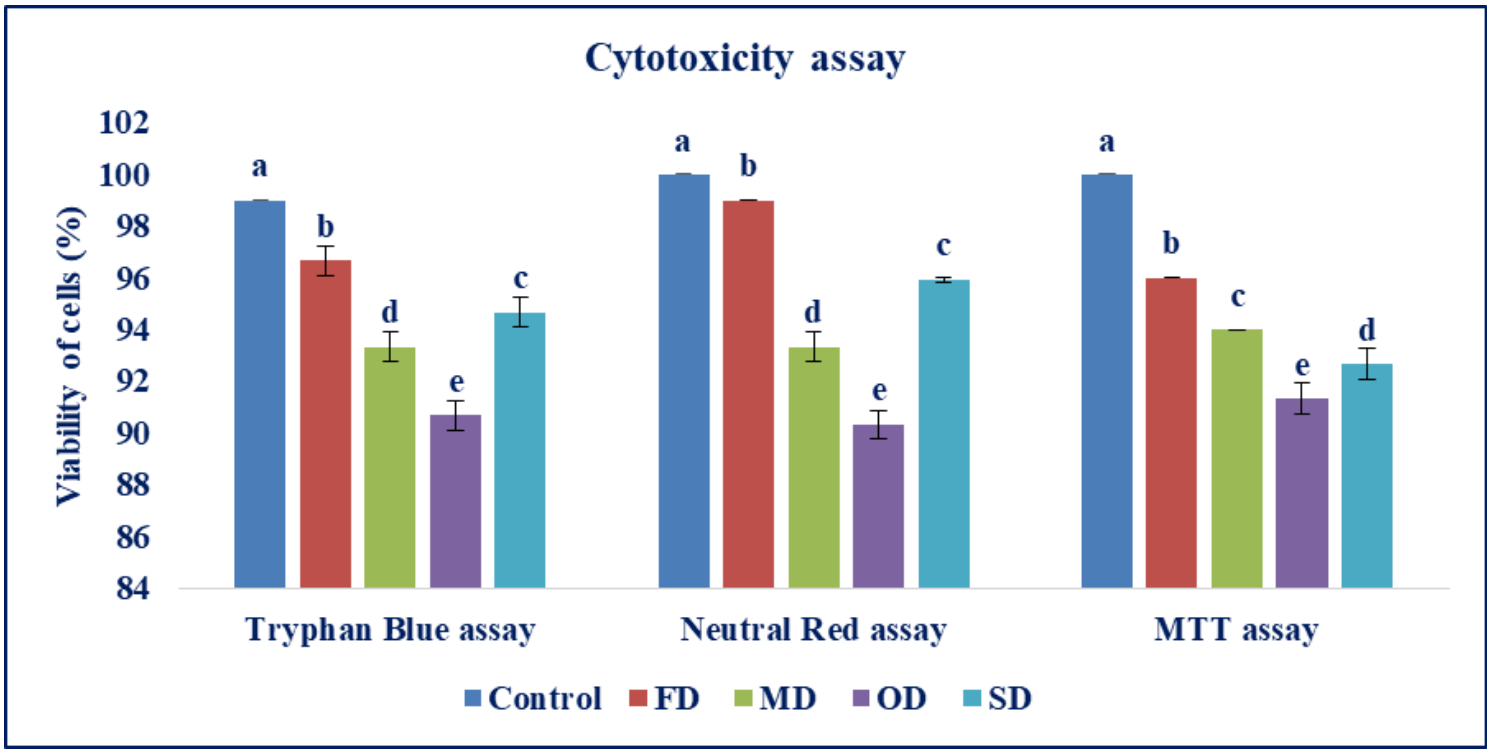


Figure 4

Figure 2 Antimicrobial activity of MPP obtained by different drying methods against selected food-borne pathogens *(S. aureus, B. cereus, P. fragi,* and *S. typhi)* measured by agar well diffusion assay