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Eco-Friendly Antimicrobial Food Packaging Films Based on PVA/Starch/Glycerol Blended with Frankincense Ethanolic Extract

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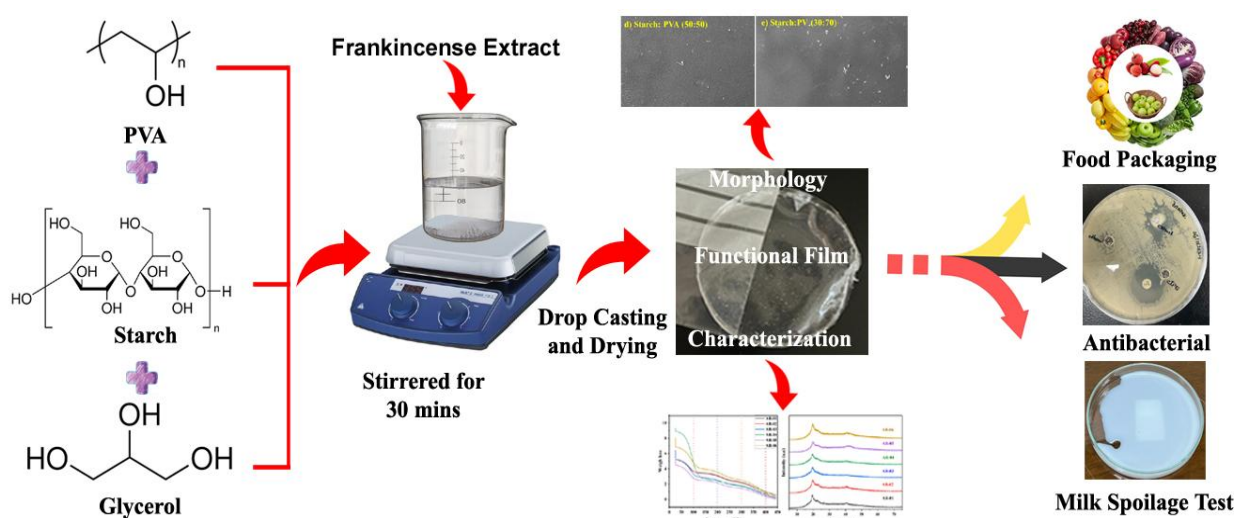
Abstract

The development of biodegradable packaging materials is a critical research priority driven by the environmental damage caused by non-degradable petroleum-based plastics. In this study, eco-friendly active packaging films were fabricated by incorporating varying concentrations of frankincense ethanolic extract (0-0.5 g per 50 g film-forming solution, samples AR-01 to AR-06) into a PVA/starch/glycerol matrix (70:30 wt/wt PVA/starch, 10 wt% glycerol). Structural and morphological characterizations were performed by FTIR, XRD, SEM-EDX, and TGA/DSC. SEM analysis demonstrated that increasing extract concentration progressively improved film homogeneity: the control film (AR-01) exhibited a rough, irregular surface, whereas AR-06 (0.5 g extract) showed the smoothest morphology, validated by a shift in O-H band position in the FTIR spectrum and a reduction in XRD peak intensity from AR-02 to AR-06, consistent with reduced crystallinity through hydrogen bonding interactions. TGA showed that at 350°C, AR-06 retained 42% residual mass compared with 38% for the control. Water absorption capacity (WAC) decreased from 81.6% in the control (AR-01) to 57.4% in AR-06, reflecting increased hydrophobic character of the matrix at higher extract loading. Tensile strength and elongation at break increased

concurrently from 2.752 MPa/6.241% (AR-01) to 3.941 MPa/10.235% (AR-06). Antimicrobial testing against *Pseudomonas aeruginosa* and *Bacillus subtilis* confirmed that AR-06 produced the largest zones of inhibition among all formulations. A food spoilage test using pasteurized milk sealed at 25°C for 48 h showed that AR-06 maintained a pH of 7.0 versus 6.0 for the unsealed control, with no visible signs of spoilage. Soil burial tests confirmed progressive biodegradation, with AR-06 exhibiting the highest weight loss after four weeks. These results demonstrate that frankincense ethanolic extract functions as a multifunctional additive in PVA/starch/glycerol blends, simultaneously reinforcing mechanical integrity, improving moisture resistance, and conferring antimicrobial activity.

Keywords: Polyvinyl alcohol (PVA); starch; frankincense ethanolic extract; glycerol; active food packaging; antimicrobial films; biodegradable packaging.

Graphical Abstract



1. Introduction

The large-scale manufacture of synthetic, petroleum-based plastics has created a persistent environmental crisis. With over 368 million metric tons produced annually, and approximately 79% of plastic waste accumulating in landfills or natural ecosystems, the consequences range from marine pollution to habitat destruction [1]. The degradation of synthetic plastics into microplastics constitutes an additional and growing threat to human health, as these particles have been detected in human blood and biological tissues [2]. In response, there is growing legislative and scientific pressure to develop biodegradable packaging materials aligned with circular economy principles [3, 4]. Biodegradable films derived from renewable resources offer a viable alternative to mitigate environmental impact while potentially extending the shelf life of food products [2]. Beyond providing a simple physical barrier, modern food preservation demands active antimicrobial packaging that can prevent spoilage by pathogens and maintain the sensory and safety attributes of the contents [5, 6]. Polyvinyl alcohol (PVA) has emerged as a primary matrix polymer for sustainable packaging due to its excellent film-forming properties, high tensile strength, chemical resistance, low oxygen permeability, and biodegradability in aerobic soil environments [2]. As a non-toxic, water-soluble polymer, PVA is frequently blended with starch to reduce material cost and increase the overall biodegradability of the composite [1, 2]. Starch is a renewable, low-cost polysaccharide composed of amylose and amylopectin, capable of forming a continuous matrix [1]. However, pure starch films are characterized by poor water resistance and high brittleness, properties that restrict their direct industrial application [2, 4]. Glycerol serves as a plasticizer in these biopolymer blends by inserting into the polymer chain network, disrupting intermolecular hydrogen bonding between PVA and starch chains, and lowering the glass transition temperature. The result is improved elongation at break and reduced stiffness, enabling the film to withstand

mechanical deformation without fracture [2, 7]. However, plasticization alone does not address the requirement for active antimicrobial functionality in modern food packaging [1]. Frankincense is an oleoresinous exudate obtained from trees of the genus *Boswellia* (family Burseraceae). The ethanolic extract of frankincense resin is rich in non-volatile bioactive compounds, primarily boswellic acids (triterpenic acids) and terpenoids, including the potent pharmacologically active compound 3-O-acetyl-11-keto- β -boswellic acid (AKBA). This composition distinguishes the ethanolic extract clearly from frankincense essential oil, which is obtained by hydro-distillation and contains predominantly volatile monoterpenes and diterpenes rather than the high-molecular-weight triterpenoids responsible for the extract's strong biological activity [8, 9]. Saied et al. (2020) prepared alginate-based films incorporating frankincense essential oil (FEO) via hydro-distillation of *Boswellia carterii* resin and confirmed by FTIR that the addition of FEO did not cause covalent changes in the alginate matrix, while SEM-EDX verified the homogeneous distribution of FEO within the film [8]. The FEO-loaded films showed antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans* [8]. Shah et al. (2025) reported that frankincense oleoresin incorporated into pectin/sodium alginate composite films inhibited *P. aeruginosa* and showed relevant structural integration into the biopolymer network [5]. While PVA/starch blend films have been extensively reviewed [2], and plant extracts have been integrated into starch-based packaging systems [1, 3], no published study has specifically examined frankincense ethanolic extract as a functional additive in a PVA/starch/glycerol matrix. Previous frankincense-related film studies have focused either on essential oil [8] or oleoresin in non-PVA matrices [5]. The present work addresses this gap by systematically varying frankincense ethanolic extract concentration (0-0.5 g per 50 g film solution) and characterizing the resulting structural, thermal, and functional changes.

The objective of this study is to fabricate and characterize eco-friendly active food packaging films based on a PVA/starch/glycerol matrix (70:30 wt/wt) blended with frankincense ethanolic extract. The films are evaluated for morphological, structural, and thermal properties, as well as antimicrobial efficacy against *Pseudomonas aeruginosa* and *Bacillus subtilis*, water absorption, mechanical strength, food spoilage performance, and soil biodegradability.

2. Materials and Method

2.1. Materials

Polyvinyl alcohol (PVA, 98% hydrolyzed, MW 115,000) was purchased from Loba Chemie Pvt. Ltd. Corn starch (MW 342.297) was sourced from Kanto Chemical Co., Inc. Glycerol (analytical grade 99.5%) was obtained from Sigma-Aldrich. Frankincense resin was collected from Salalah, Dhofar region of the Sultanate of Oman. Distilled water was used throughout the study as the primary solvent.

2.2. Preparation of Frankincense Ethanolic Extract

The powdered resin (25.0 g) was subjected to continuous Soxhlet extraction with 500 mL of 95% ethanol for 4 h. The solvent was evaporated using a rotary evaporator (Büchi Rotavapor R-200) at 40°C under reduced pressure to yield 6.7 g of a yellowish viscous extract (extraction yield: 26.8% w/w). The concentrated extract was stored at 4°C in a sealed amber glass vial until use. The Soxhlet ethanolic extract is expected to be enriched in non-volatile compounds, including boswellic acids and terpenoids, consistent with the polarity of the 95% ethanol solvent.

2.3. Preparation of PVA/Starch films

PVA/starch blend films were prepared by the solution casting method using different PVA-to-starch weight ratios: 100/0, 0/100, 70/30, 50/50, and 30/70 wt/wt%, as presented in [Table S-1 \(Supplementary information\)](#). The calculated amount of polyvinyl alcohol (PVA) was dissolved in 100 mL of distilled water at 85–90 °C under continuous stirring for 1 h until a clear and

homogeneous solution was obtained. The solution was then cooled to room temperature while stirring was maintained. Separately, the required amount of starch was dispersed in distilled water and heated under continuous stirring until complete gelatinization occurred. For the preparation of PVA/starch blend films, the gelatinized starch solution was gradually added to the PVA solution under continuous stirring to obtain a uniform film-forming mixture. The final solution was cast onto a clean glass plate and dried at 40 °C for 48 h to ensure complete solvent evaporation. After drying, the films were carefully peeled from the glass surface and stored for further characterization. Among the prepared formulations, the PVA/starch film with a 70/30 ratio exhibited the most favorable mechanical and physical properties, particularly higher elongation and elasticity.

2.4.Preparation of PVA/Starch/Glycerol Films Incorporated with Frankincense Extract

The film composition was optimized based on a preliminary screening of PVA/starch ratios (**Table S-1**). The 70:30 wt/wt ratio showed the most favorable mechanical properties and was selected for all extract-loaded formulations. For each film batch, 7.87 g of PVA (98% hydrolyzed, MW 115,000; Loba Chemie) and 3.37 g of corn starch (MW 342.3; Kanto Chemical) were dissolved in 130 mL of distilled water at 90–95°C under continuous stirring for 30 min. The solution was adjusted to 300 mL with distilled water and divided into six equal 50.0 g portions. Glycerol (5.0 g; Sigma-Aldrich, ≥99.5%) was added to each portion as a plasticizer. Frankincense ethanolic extract was then incorporated at 0, 0.1, 0.2, 0.3, 0.4, and 0.5 g to obtain formulations AR-01 through AR-06, respectively (**Table S-2**). Each solution was thoroughly mixed and cast onto clean Petri dishes. The films were dried at 60°C until complete solvent evaporation, then carefully peeled and stored at ambient conditions prior to characterization. The complete synthesis diagram is shown in **Figure 1**.

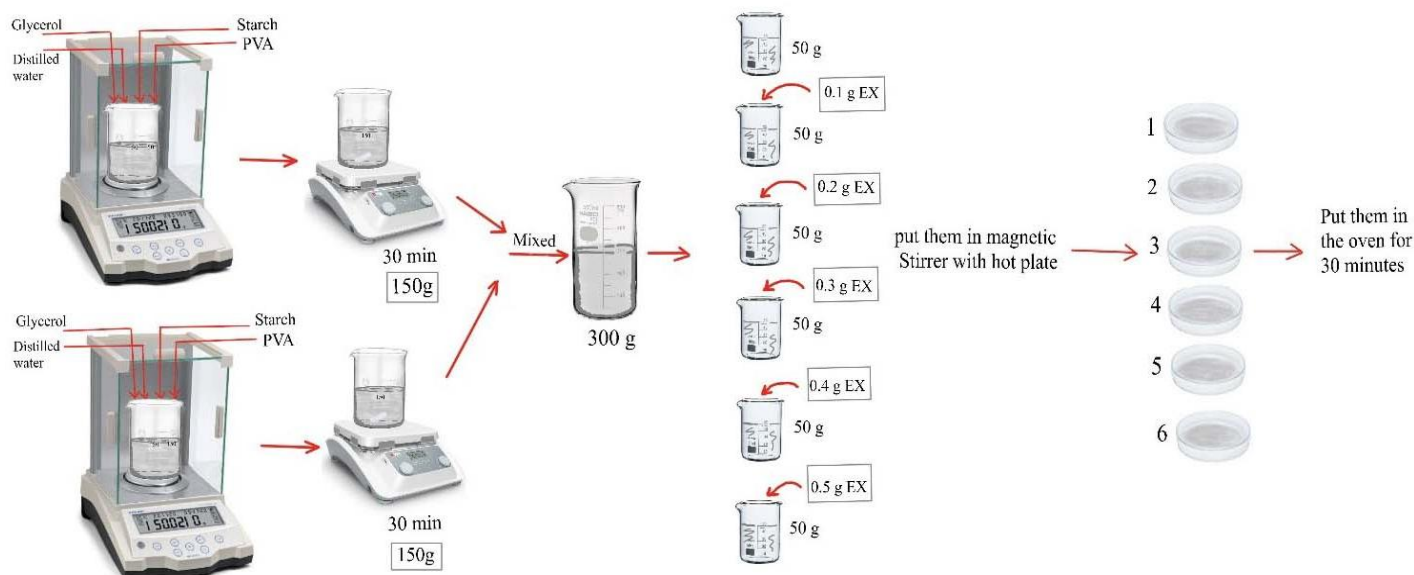


Figure 1: Fabrication scheme of PVA/starch antimicrobial biodegradable films

2.5.Characterization of formulated films

Surface Morphology and Elemental Composition: Surface texture and elemental weight percentages were analyzed using Scanning Electron Microscopy (SEM: Axia ChemiSEM System, Thermo-Fisher Scientific) coupled with Energy Dispersive X-ray Spectroscopy (EDX). Samples were sputter-coated with platinum prior to imaging. Fourier Transform Infrared (FTIR) spectroscopy was conducted using a Bruker ALPHA II spectrometer equipped with a Platinum ATR accessory. Spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} . X-ray Diffraction (XRD) patterns were obtained using a PANalytical X'Pert PRO diffractometer with Cu K radiation ($\lambda=1.54 \text{ \AA}$). The 2θ range spanned from 5° to 140° with a step size of 0.5° . Thermogravimetric Analysis (TGA) was performed using a Seiko EXSTAR TGA/DTA 6300. Samples were heated from 0 to 700 $^\circ\text{C}$ at a rate of 10 $^\circ\text{C}/\text{min}$ under a nitrogen atmosphere. Instron test unit was used for measuring film mechanical properties, using the procedure already described in the literature [10]. Three replicates per formulation were measured. TS was calculated as the

maximum force at break divided by cross-sectional area (MPa), and EAB as the percentage extension relative to the initial gauge length.

2.6.Functional Evaluation

For the evaluation of functional and protective properties of formulated films, the Anti-bacterial, food spoilage, water absorption, and soil burial degradation tests were conducted. Antimicrobial activity of the formulated films was assessed against *Pseudomonas aeruginosa* (Gram-negative) and *Bacillus subtilis* (Gram-positive) using the agar disk diffusion method. Film samples were cut into standard discs and placed on agar plates inoculated with a standardized bacterial suspension. Plates were incubated at 37°C for 24 h. Zones of inhibition were measured in millimeters. Three replicates per organism per film formulation were performed. [11]. A food spoilage test was conducted to determine whether these active films could preserve food quality. From the local market, the pasteurized milk pack (milk pack) was bought. Square-cut films were used to seal the 10 mL vials after the milk had been added. The milk was tested for freshness 48 hours later [12]. Water absorption capacity (WAC) of the prepared films was determined using the weight-gain method. Briefly, film specimens were dried to constant weight, and their initial dry weight was recorded as W_0 . The samples were then immersed in distilled water at room temperature for 24 h. After immersion, the films were removed, gently blotted with filter paper to remove surface water, and immediately weighed as W_t . The WAC was calculated using the following equation. The increase in weight indicated the amount of water absorbed, helping to assess the film hydrophilicity, swelling behavior, and suitability for various applications [13, 14]. The swelling behavior (SB) in starch/PVA blend film was calculated as:

$$\text{WAC (\%)} = \left(\frac{W_t - W_0}{W_t} \right) \times 100 \quad (1)$$

Where W_t is the weight of starch/PVA blend film after absorption, and W_0 is the first dry weight of starch/PVA blend film. WAC (%) Water Absorption Capacity. The soil burial degradation test was conducted by burying the films in fertile soil naturally exposed to the environment. The samples were cut into dumbbell shapes and buried 10 cm deep in the soil. After four weeks, the samples were carefully removed, gently washed with distilled water to eliminate any soil residues, and weighed to determine weight loss, indicating the extent of degradation [15]. The biodegradability of the formulated films was determined by soil burial tests (expressed as weight loss percentage %) and was calculated as:

$$\text{Weight Loss (\%)} = \left(\frac{W_0 - W_t}{W_0} \right) \times 100 \quad (2)$$

Where W_0 (before soil burial) is the initial dry weight of formulated films, and W_t (after being buried in soil) is the weight of formulated films at time t .

3. Results and discussion

3.1. Morphological Characterization

SEM analysis: SEM, FTIR, XRD, and mechanical characterisation were performed on five representative formulations (AR-01, AR-02, AR-04, AR-05, AR-06); AR-03 was excluded as its properties were intermediate to AR-02 and AR-04 and did not provide additional insight into the concentration-dependent trend. The SEM images (**Figure 2a-e**) reveal a concentration-dependent improvement in film surface uniformity with increasing frankincense ethanolic extract content. The control film (AR-01) exhibits a rough surface with irregular texture, characteristic of a base PVA/starch matrix with incomplete polymer interdiffusion. EDX analysis confirms that AR-01 is composed exclusively of 48.8 wt% carbon and 51.2 wt% oxygen (**Table S-3 + Figure S-1**), consistent with the chemical structure of the PVA/starch blend (no heteroatoms from exogenous compounds). Sample AR-02 (0.1 g extract) shows a slightly smoother surface at

comparable elemental composition (**Table S-4 + Figure S-2**: 48.8% C, 51.2% O), suggesting that at this loading, the extract is present but does not yet alter the bulk elemental profile. At intermediate extract concentrations, samples AR-04 and AR-05 display spherical microstructural features, indicative of partial phase separation when the concentration of non-polar triterpenoid components exceeds local solubility in the hydrophilic PVA/starch matrix. EDX analysis of AR-04 (**Table S-5 + Figure S-3**) shows a shift to 43.4% C and 56.6% O, while AR-05 (**Table S-6 + Figure S-4**) introduces 4.5% nitrogen, confirming the incorporation of nitrogen-containing compounds from the extract into the polymer matrix. At the highest extract loading, AR-06 (0.5 g extract) presents the most uniform and smooth surface among all formulations, with no visible cracks or wrinkles. EDX analysis of AR-06 (**Table S-7 + Figure S-5**) indicates the highest degree of elemental incorporation.

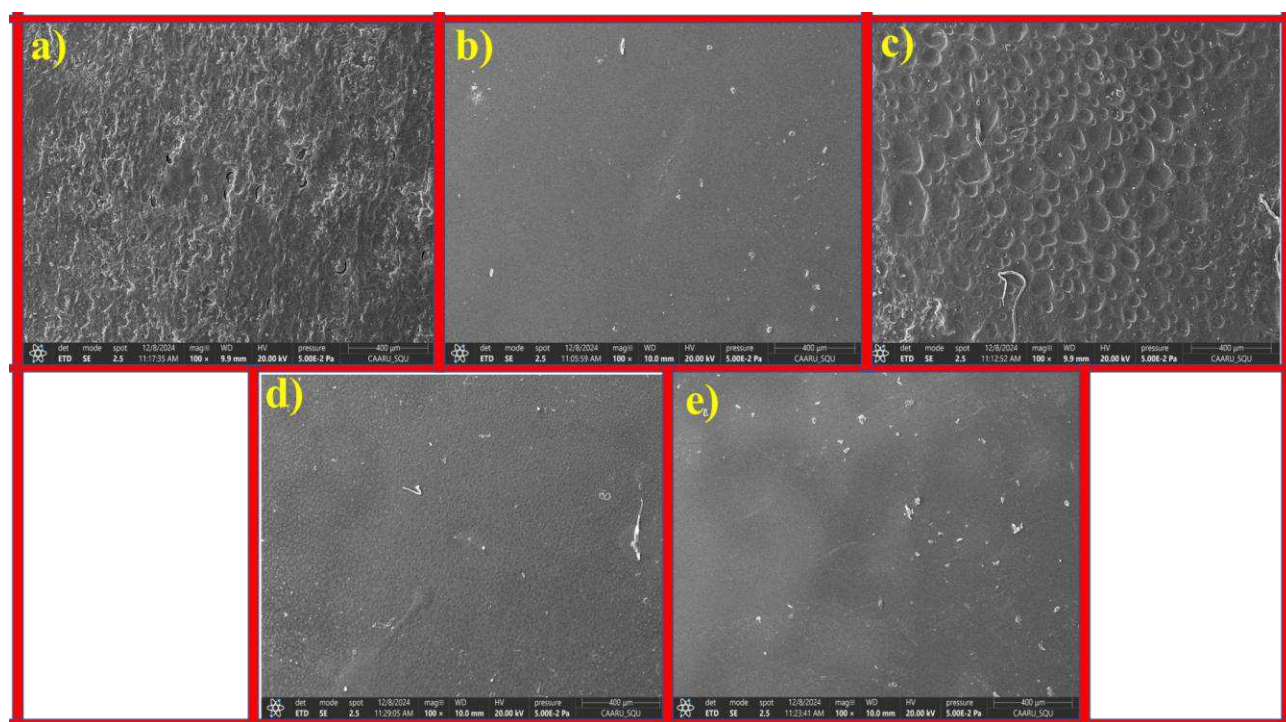


Figure 2: SEM images of (a) AR-01, (b) AR-02, (c) AR-04, (d)AR-05, (e) AR-06

The improvement in surface homogeneity at high extract concentration is consistent with the formation of a more cohesive polymer network, driven by hydrogen bonding and physical interactions between the hydroxyl groups of PVA and starch and the boswellic acid carboxyl and hydroxyl functionalities. Analogous morphological improvements following plant extract incorporation into biopolymer films have been reported using hawthorn berry extract in alginate matrices [16] and green tea extract in starch/PVA-PEG copolymer films [3].

3.2. Structural characterization

FTIR Spectra: The FTIR spectra of the PVA/starch/glycerol films (**Figure 3**) exhibit characteristic absorption bands of the polymer matrix: a broad O-H stretching peak between 3200 and 3500 cm^{-1} , aliphatic C-H stretching at approximately 2900 cm^{-1} , and C-O and C-O-C stretching vibrations at approximately 1000 and 1150 cm^{-1} , respectively. These assignments are consistent with the reported spectra of PVA/starch blends [2]. The control film AR-01 shows a standard plasticized PVA/starch spectral profile. As frankincense ethanolic extract concentration increases from AR-02 to AR-06, progressive changes are observed in peak intensity across the fingerprint region. In AR-06, a shift in the O-H stretching band relative to the control indicates interaction between the extract and polymer hydroxyl groups. No new absorption peaks appear in any formulation, confirming that the frankincense ethanolic extract does not induce covalent bond formation with the polymer chains. This behavior is consistent with physical blending rather than chemical crosslinking, and is supported by Saied et al. (2020), who similarly reported that frankincense essential oil (FEO) incorporated into alginate films did not produce new peaks in FTIR spectra, with only intensity changes observed for existing bands [8]. Rana et al. (2026) also reported broadening and shifting of O-H peaks upon green tea extract incorporation into

starch/PVA-PEG copolymer films, attributing this to intermolecular hydrogen bonding between polyphenolic extract components and polymer chains, a mechanism directly applicable to the boswellic acid functionalities in frankincense ethanolic extract [3]. Shah et al. (2025) is reported to have identified characteristic peaks of frankincense at C=O stretching and aliphatic C-H positions integrated into biopolymer spectra through physical blending [5].

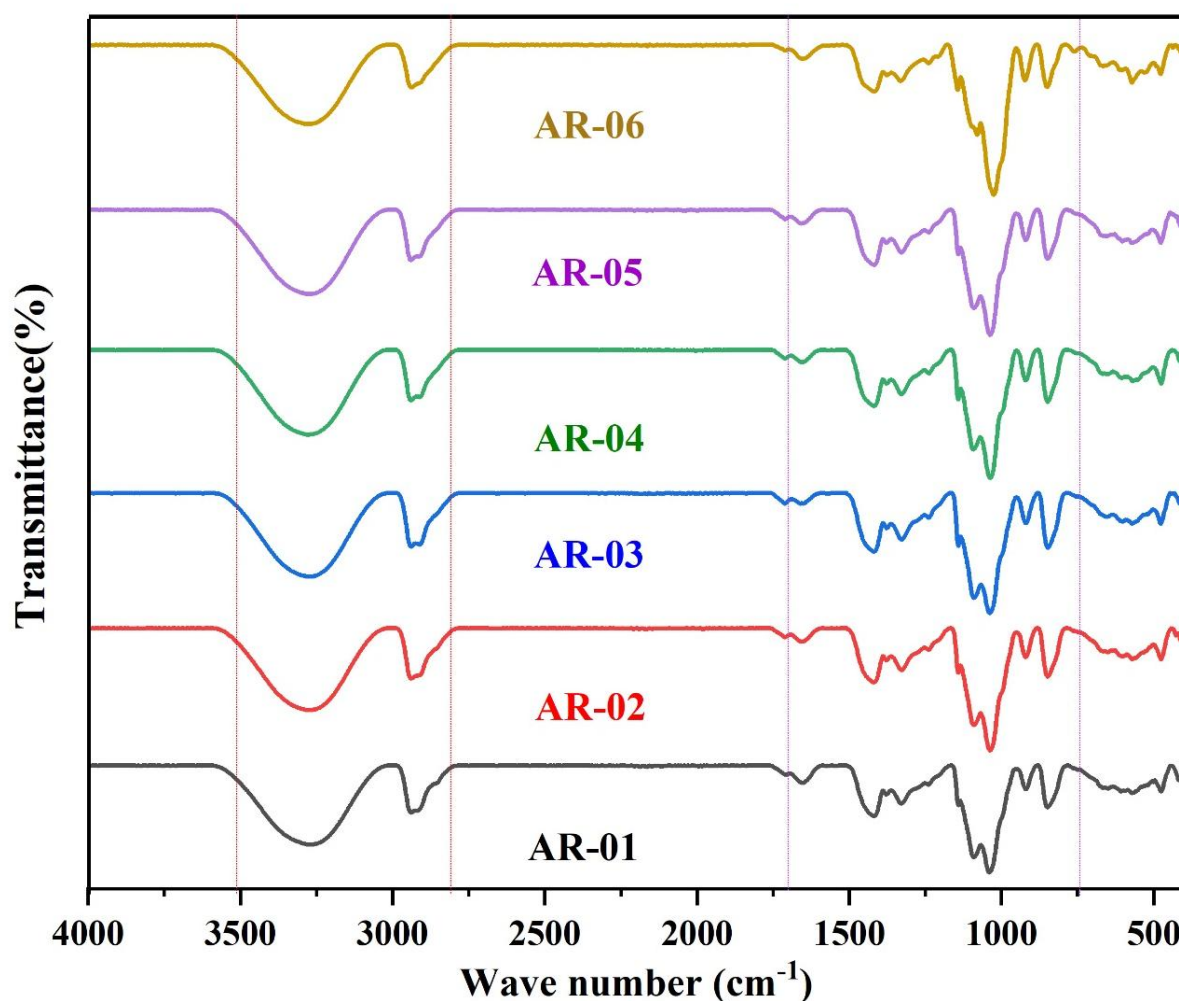


Figure 3: FTIR spectra of (a) AR-01, (b) AR-02, (c) AR-04, (d)AR-05, (e) AR-06

XRD Analysis: The XRD diffractograms (**Figure 4**) of all formulations exhibit a broad diffraction peak centered at $19\text{--}21^\circ$ (2θ), characteristic of the semi-crystalline PVA/starch matrix. The control film AR-01 shows the most intense diffraction peak at 19.70° , reflecting the highest degree of crystallinity in the absence of extract. Incorporation of frankincense ethanolic extract at increasing concentrations (AR-02 to AR-06) results in a progressive decrease in peak intensity and broadening of the diffraction band. Quantitatively, the peak intensity decreases from 60.22 counts in AR-02 to 39.10 counts in AR-06. This reduction in crystallinity is attributed to physical interactions and hydrogen bonding between the boswellic acids and terpenoid components of the frankincense extract and the hydroxyl groups of PVA and starch, which disrupt regular polymer chain packing without altering the overall structural framework of the matrix. Rana et al. (2026) confirmed an analogous amorphization in starch/PVA-PEG copolymer films incorporating green tea extract, describing the transition as reflecting strong interfacial interactions between extract and polymer components[17, 18]. The fundamental PVA/starch diffraction pattern is retained in all samples, confirming that the basic polymer network is preserved. The observed decrease in crystallinity differs from results reported by Shah et al. (2025) and Hosseini et al. (2016), who found that frankincense oleoresin or oregano essential oil increased crystallinity in their respective matrices [5, 19]. This discrepancy may reflect differences in extract composition: the ethanolic extract used here contains predominantly non-volatile polar compounds (boswellic acids), which interact more strongly with the hydroxyl-rich PVA/starch matrix than the volatile monoterpenes present in essential oils

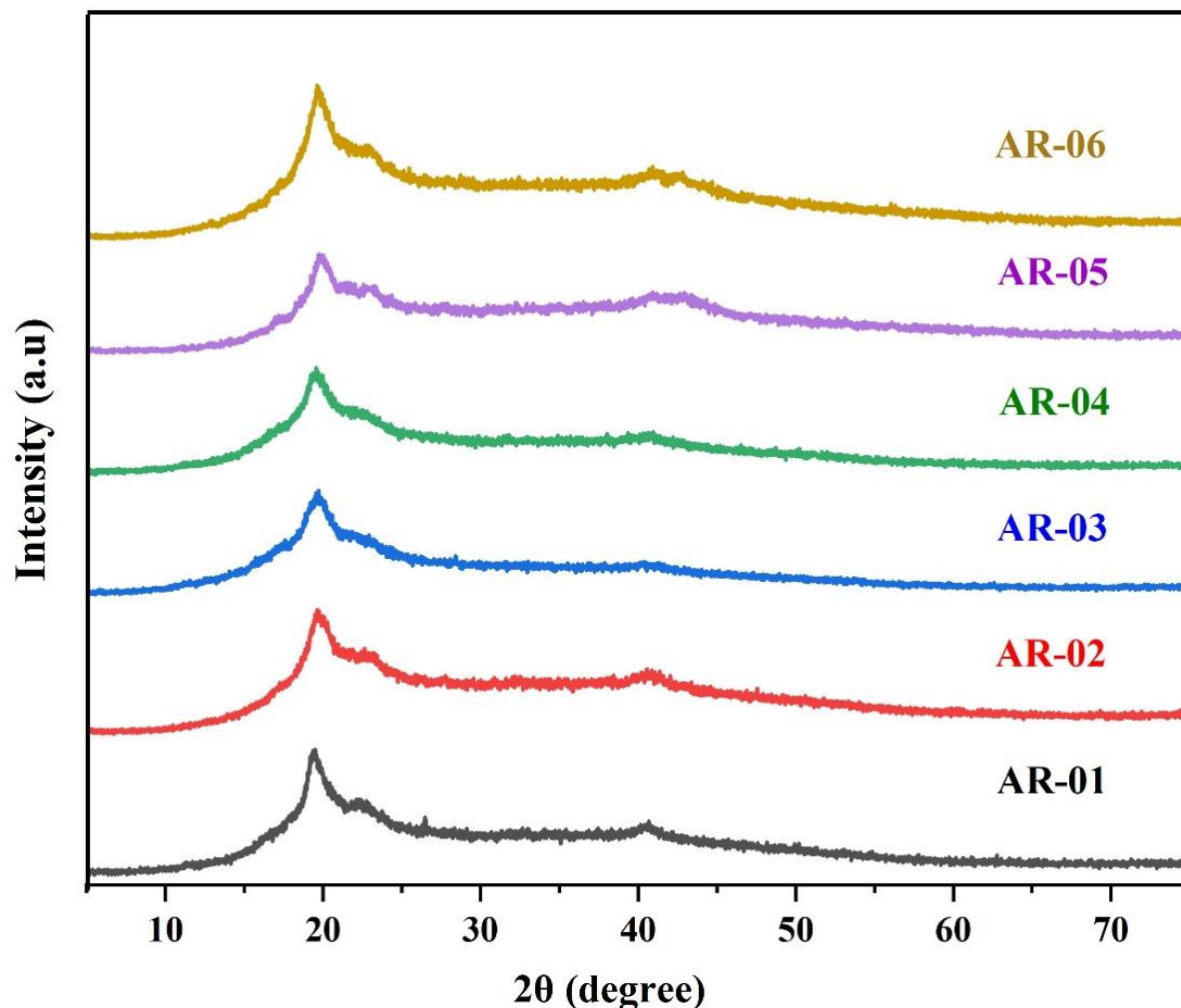


Figure 4: XRD analysis patterns of PVA/ starch (AR-01) with plant extract films (AR-02, AR-03, AR-04, AR-05, AR-06) sample.

The TGA thermograms (**Figure 5; Figure S-6 to S-11**) show a consistent multi-step degradation profile across all formulations (AR-01 to AR-06), with three identifiable decomposition regions. The first stage, near 100°C, corresponds to the loss of physically adsorbed moisture and volatile organic compounds [20]. Extract-loaded films (AR-04 to AR-06) show slightly more pronounced mass loss in this region than the control, consistent with the hygroscopic functional groups of boswellic acid compounds. At 350°C, the control film (AR-01) had degraded by 62%, retaining

38% residual mass. In contrast, AR-06 demonstrated improved thermal resistance, degrading by only 58% at this temperature and retaining 42% residual mass. The enhanced thermal resistance of the extract-loaded films is consistent with increased intermolecular resistance to thermal decomposition, [3] [21]. Rana et al. (2026) reported analogous improvements in thermal stability following green tea extract incorporation into starch/PVA-PEG films, attributed to reinforcement and reduced chain mobility [3]. Kumar et al. (2021) similarly reported increased thermal stability upon pineapple peel extract incorporation in PVA/starch films [22].

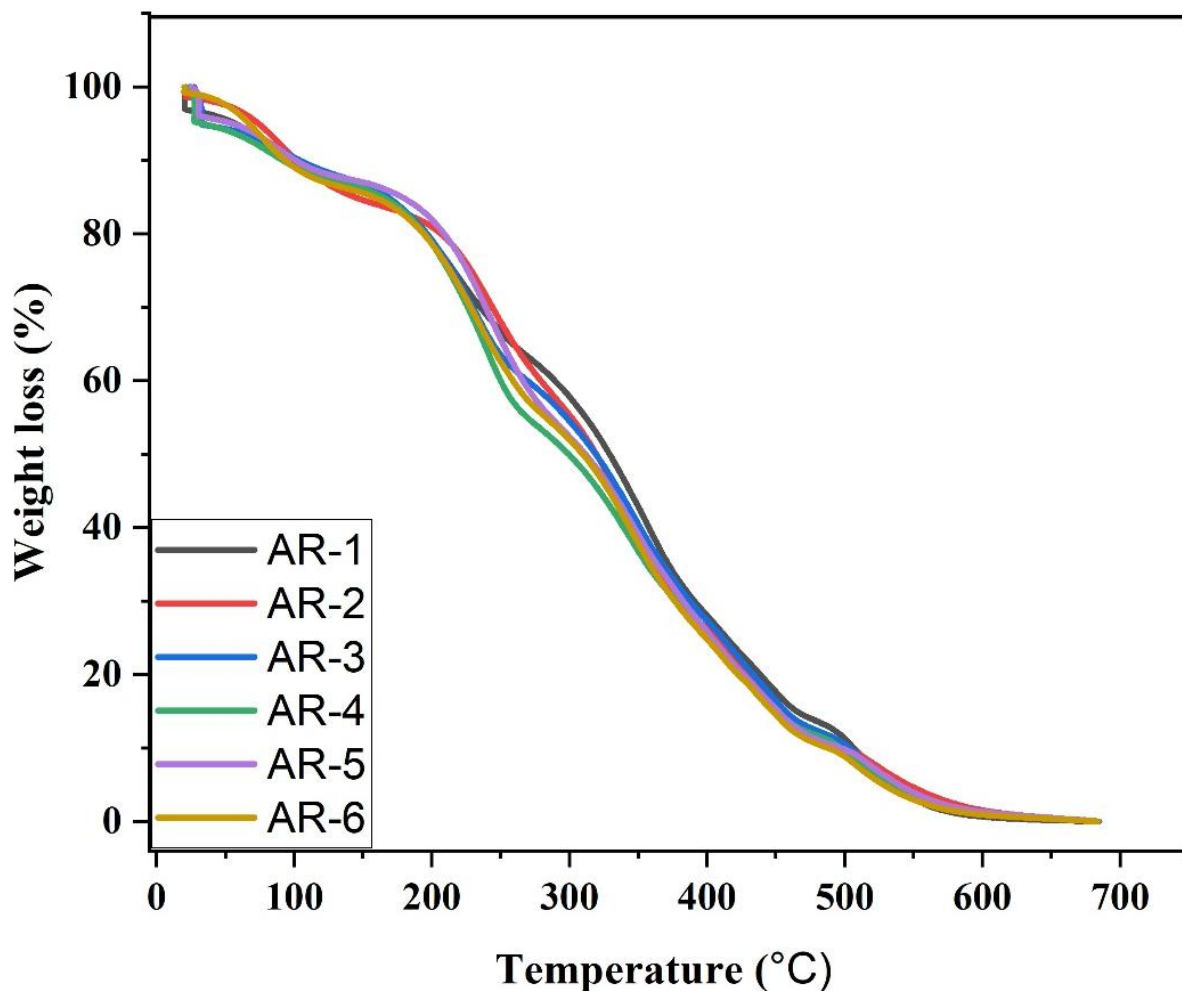


Figure 5: TGA spectra of PVA/ starch (AR-01) with plant extract films (AR-02, AR-03, AR-04, AR-05, AR-06) sample.

4. Evaluation of Functional and Protective Properties

4.1. Water absorption capacity test

The water absorption capacity (WAC) values for AR-01 through AR-06 are summarized in **Figure 6** and **Table S-8**. As frankincense ethanolic extract concentration increases, WAC decreases monotonically: AR-02 = 80.1%, AR-03 = 68.3%, AR-04 = 67.4%, AR-05 = 66.2%, and AR-06 = 57.4%, representing a 24.2 percentage-point absolute reduction from the control (AR-01 = 81.6%), equivalent to a 29.7% relative reduction. The progressive decrease in WAC is attributed to the hydrophobic character of the terpenoid and boswellic acid components of the extract, which reduces the overall polarity of the polymer matrix. When integrated into the PVA/starch network, these non-polar moieties restrict the access of water molecules to the abundant hydroxyl groups of PVA and starch, reducing swelling. Deng et al. (2024) demonstrated that blending starch with PVA creates hydrophobic regions through polymer interactions that reduce water affinity [2]. Rana et al. (2026) reported a significant decrease in moisture content upon 0.5% green tea extract addition to starch/PVA-PEG films, from 22.28% to 17.16%, attributing the reduction to hydrophobic constituents of the extract [3]. Tarique et al. (2021) further showed that glycerol hydrogen-bonding interactions with starch modify the accessibility of polymer hydroxyl groups to water [7].

4.2. Mechanical Properties:

The tensile strength (TS) and elongation at break (EAB) data (**Figure 7 + Table S-9**) show a progressive enhancement with increasing extract concentration. The control film (AR-01) has a TS of 2.752 MPa and EAB of 6.241%. Upon the addition of the plant extract, a steady improvement is observed: sample AR-02 shows an increase to 2.852 MPa (TS) and 7.356% (EAB),

culminating in the optimal AR-06 formulation, which achieves a TS of 3.941 MPa and an EAB of 10.235%.

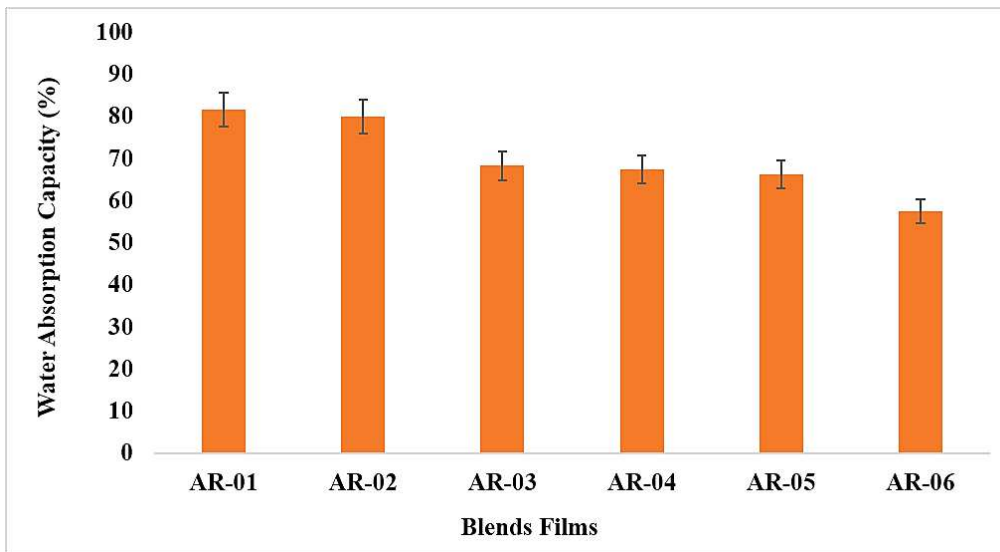


Figure 6: Water absorption of different PVA/starch with plant extract blends (AR-01, AR-02, AR-03, AR-04, AR-05, AR-06).

The simultaneous increase in both TS and EAB is mechanistically attributed to the formation of a denser intermolecular network driven by hydrogen bonding and physical interactions between the carboxyl and hydroxyl groups of boswellic acids in the extract and the hydroxyl-rich PVA/starch backbone. This reinforcement allows more effective stress distribution within the film while preserving chain mobility, yielding both stronger and more extensible films. Rana et al. (2026) reported a peak TS of 4.7 ± 0.3 MPa in starch/PVA-PEG films with 0.5% green tea extract, compared with a blank film TS of 2.2 ± 0.9 MPa - a proportional enhancement consistent with the 43% increase in TS observed in this study (2.752 to 3.941 MPa) [3]. Deng et al. (2024) confirmed that PVA-starch blending creates a novel structural network that enhances film physical and mechanical properties [2].

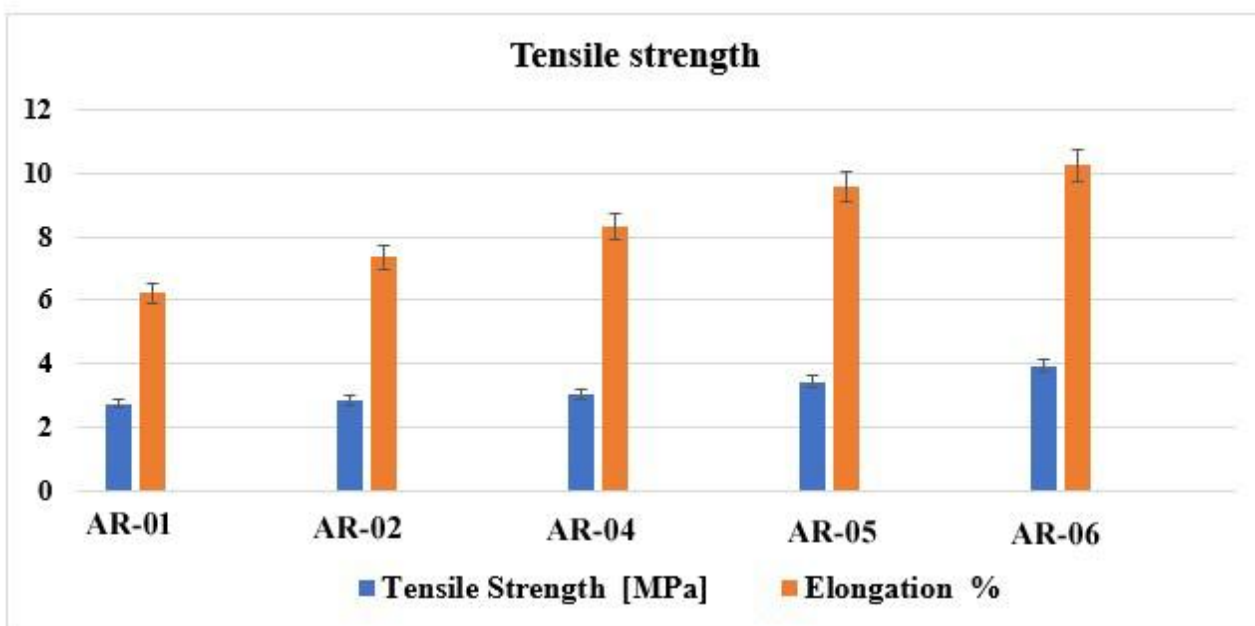


Figure 7: Mechanical Properties (Tensile Strength and Elongation) of PVA/starch with plant extract blends (AR-01, AR-02, AR-03, AR-04, AR-05, AR-06).

4.3. Antimicrobial test

The antimicrobial activity of the PVA/starch/glycerol films against *Pseudomonas aeruginosa* and *Bacillus subtilis* (**Figure 8**) shows that the control film AR-01 and the lowest-extract-concentration film AR-02 produce no measurable inhibition zones. As extract concentration increases, antibacterial efficacy becomes progressively more pronounced, with AR-06 (0.5 g extract) producing the largest inhibition zones against both bacterial strains. The antimicrobial mechanism of frankincense ethanolic extract is proposed to involve disruption of bacterial membrane integrity, leading to increased membrane permeability, leakage of intracellular metabolites, and eventual cell death. This mechanism has been experimentally confirmed for acetyl-11-keto- β -boswellic acid (AKBA), the key bioactive triterpene present in *Boswellia* resin through propidium iodide uptake and cellular leakage assays [23]. The activity of the whole ethanolic extract against both the Gram-positive *B. subtilis* and the Gram-negative *P. aeruginosa*

observed in this study is consistent with Al-Saban et al.(2025), who demonstrated that a Soxhlet ethanolic extract of *Boswellia sacra* produced measurable inhibition zones against *Bacillus cereus* and *P. aeruginosa*, noting that the crude extract contains multiple terpenoid components with complementary activity spectra beyond that of isolated AKBA [24]. Although direct mechanistic confirmation was not performed in this study, the concentration-dependent pattern of inhibition with AR-01 and AR-02 showing no inhibitory zones and AR-06 producing the largest, is consistent with a membrane-targeting mechanism requiring a threshold concentration of bioactive compounds to achieve permeability disruption [23, 24].



Figure 8: Antimicrobial activity for (AR-02 &AR-06) films against *Pseudomonas aeruginosa* and *Bacillus subtilis*.

4.4.Food spoilage test

The food spoilage evaluation used pasteurized milk sealed with the formulated films at 25°C for 48 h (**Figure 9**). The unsealed blank sample showed visible spoilage indicators after 48 h, including a pH drop to 6.0, consistent with the accumulation of lactic acid and other acidic

metabolites from bacterial fermentation. The control film (AR-01, no extract) showed similar spoilage progression. In contrast, all extract-loaded films (AR-02 to AR-06) delayed spoilage, with AR-06 maintaining a milk pH of 7.0 and showing no color change or visible separation. The preservation effect is attributed to the sustained release of bioactive compounds from the film into the headspace and surface of the food product, which inhibits bacterial growth and prevents the accumulation of acidic metabolites.

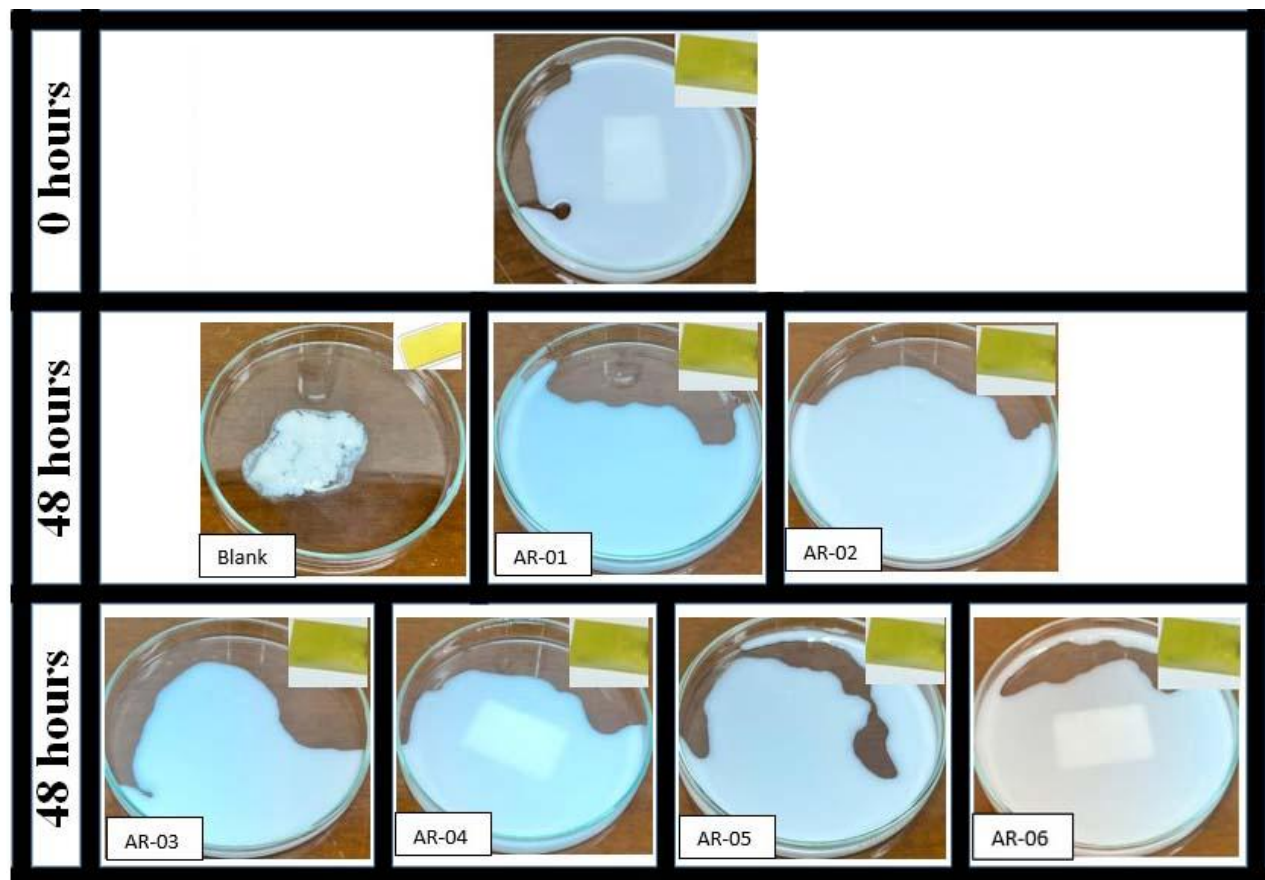


Figure 9: Photographs of pasteurized milk after 48 h with different films; from left to right are: Blank, AR-01, AR-02, AR-03, AR-04, AR-05, and AR-06 films.

Rana et al. (2026) reported that starch/PVA-PEG films incorporating green tea extract maintained better pH stability in fresh-cut apple cubes compared with blank films (STKTE 0.5%: pH 2.63 ± 0.02 vs. blank: pH 2.53 ± 0.05), supporting the pH-preservation mechanism observed here with frankincense extract in milk [3]. This also confirms that the antibacterial properties of the plant extract effectively inhibited bacterial growth, preventing milk spoilage [24].

4.5. Soil Burial Biodegradability

The soil burial test results (**Figure 10 + Table S-10**) confirm that all formulated films undergo measurable biodegradation over the four-week test period. Weight loss is calculated as $WL\% = (W_0 - W_t)/W_0 \times 100$. following the order AR-06 (38.21%) > AR-05 (37.95%) > AR-04 (37.25%) > AR-03 (28.50%) > AR-02 (25.61%) > AR-01 (24.04%), calculated from the raw data in Table S-10.

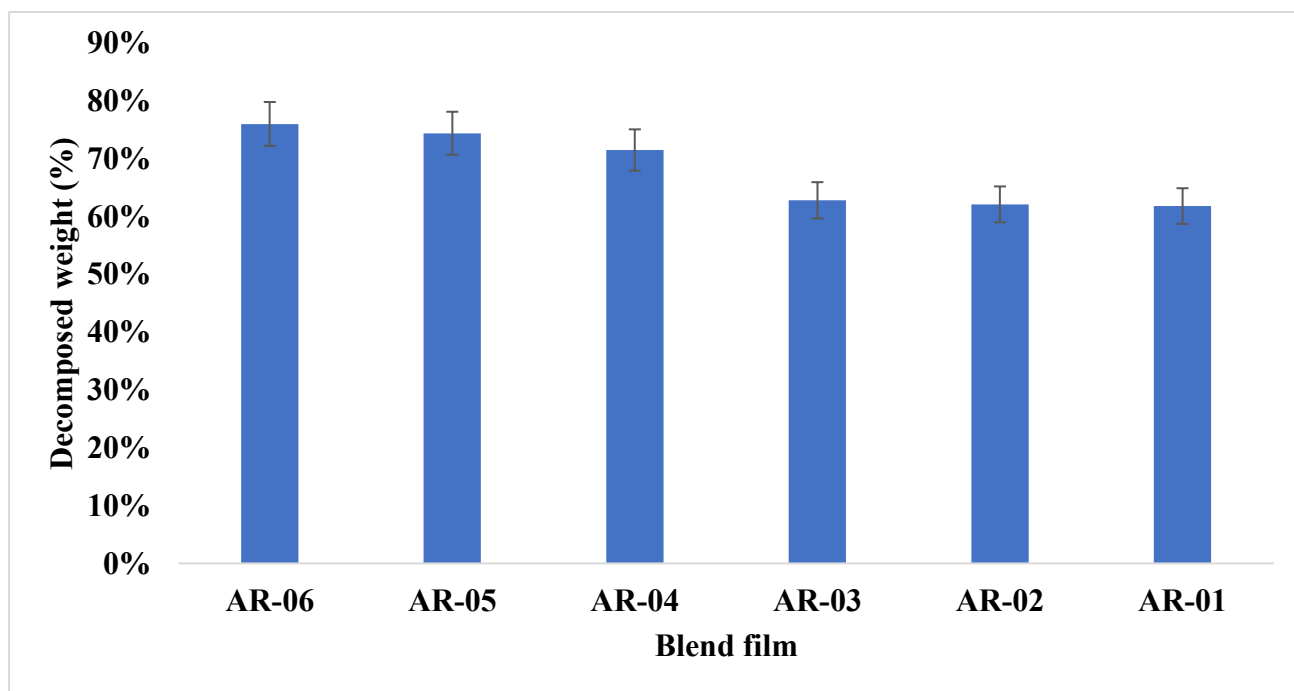


Figure 10: Percentage of decomposed weight of all samples (AR-01, AR-02, AR-03, AR-04, AR-05, and AR-06 films) after burial.

Films at extract concentrations ≥ 0.3 g (AR-04 to AR-06) showed substantially greater weight loss (~37–38%) than lower-concentration formulations (AR-01 to AR-03: 24–29%); statistical analysis (n, mean $\pm$ SD, p-value) is needed to confirm whether differences within the AR-04 to AR-06 group are significant.

Physical disintegration photographs (Figure 11) of AR-01 and AR-06 before and after four weeks of burial confirm progressive structural deterioration. The accelerated degradation of extract-loaded films, particularly AR-06, is attributed to the disruption of regular polymer chain packing by the incorporated bioactive components, which introduce microstructural heterogeneities that increase the accessibility of the matrix to soil moisture and microbial enzymes

This interpretation is supported by the SEM evidence of concentration-dependent surface morphology changes and the XRD data showing reduced crystallinity in extract-loaded films; less ordered matrices generally present more sites accessible to hydrolytic and enzymatic degradation. Deng et al. (2024) confirmed that starch/PVA blends undergo complete decomposition under natural soil conditions within weeks [2], and Rana et al. (2026) reported near-complete physical disintegration (residual area $0.6 \pm 0.6\%$) of starch/PVA-PEG/green tea extract films after only 10 days of soil burial, demonstrating the biodegradation-accelerating effect of plant extract incorporation [3]. **Table 1** shows the comparison with previous studies on the fabrication of PVA/starch-based biodegradable films, with current research work.

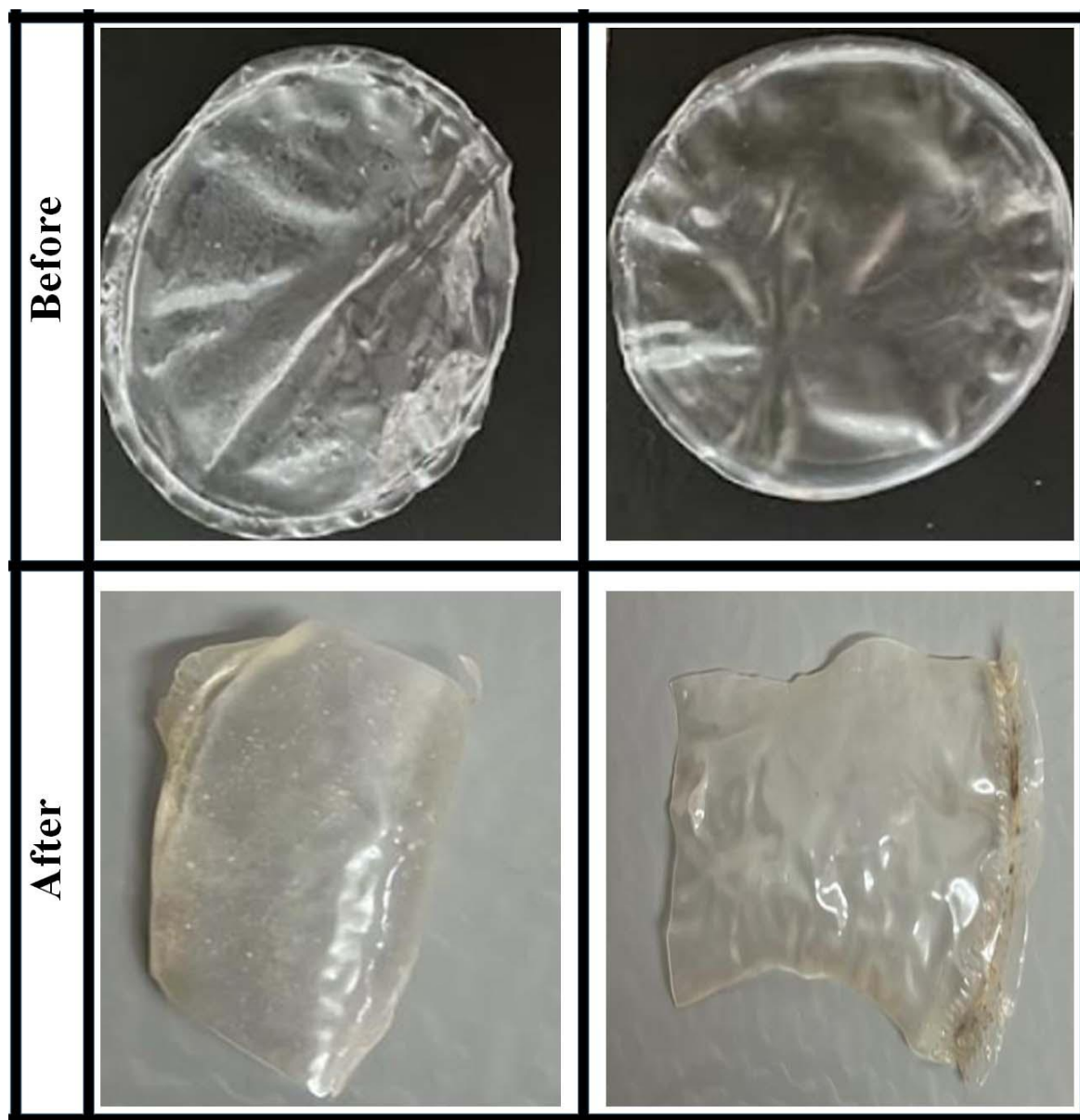


Figure 11: AR-01 and AR-06 before and after being buried for four (4) weeks

Table.1 Comparison from previous study for the fabrication of PVA/Starch-Based Biodegradable Films

Film composition	Advantages	Tensile strength (TS) MPa	Microbial Activity	Ref
PVA/Starch/Chitosan/NiO-CuO	Excellent mechanical and barrier properties	31.94	<i>S. aureus, E. coli</i>	[26]
PVA/Starch/MMT/V ₂ O ₅	Enhanced thermal conduction	-	<i>E. coli, P. aeruginosa, K. pneumoniae, S. Typhi, P. mirabilis, S. aureus, B. subtilis, C. albicans</i>	[27]
PVA/Starch/Propolis/Ant hocyanin	Intelligent packaging (pH color change)	15.9	<i>E. coli, MRSA</i>	[17]
PVA/Starch/Basil Extract	Improved shelf life	22.76	<i>E. coli, S. aureus</i>	[28]
Starch/Polyvinyl Alcohol/Citric Acid	Good transparency	46.45	<i>L. monocytogenes, E. coli,</i>	[29]
PVA/Starch/Glycerol (PSG)/CuO, ZnO	No nanoparticle leaching	-	<i>A. niger, A. calidoustus, chrysogenum, K. pneumoniae and S. aureus</i>	[30]
PVA/Starch (Bio-Composite) Films	Rapid biodegradation	-	<i>S. aureus, B.cereus, K. pneumoniae, A. fumigatus, A. niger)</i>	[31]
PVA/Starch/Cellulose Nanofiber/ lemongrass	Biodegradable film.	85	<i>S. aureus, E coli</i>	[32]
PVA/Starch/Anthocyanin /Limonene	Intelligent packaging (pH color change)	4.51	<i>B. subtilis, S. aureus, A. niger</i>	[12]
PVA/Starch/Glycerol/ Frankincense extract	water absorption capacity test (57.4%), Biodegradable film.	3.941	<i>P. aeruginosa, B. subtilis.</i>	Current work

5. Conclusion

This study demonstrates the successful integration of frankincense ethanolic extract into PVA/starch/glycerol films at concentrations from 0.1 to 0.5 g per 50 g film solution, and systematically documents the structural, thermal, and functional changes that result. SEM analysis showed that increasing extract concentration improved surface homogeneity, with AR-06 producing the smoothest film, and EDX confirming elemental changes consistent with extract incorporation. FTIR and XRD analyses confirmed that the extract interacts with the polymer matrix primarily through hydrogen bonding and physical intermolecular forces, producing band shifts and a 35% reduction in XRD peak intensity (from 60.22 to 39.10 counts, AR-02 to AR-06) without inducing new covalent species. TGA analysis showed that AR-06 retained 42% residual mass at 350°C versus 38% for the control, indicating improved thermal resistance. Functional characterization showed that WAC decreased progressively from 81.6% (AR-01) to 57.4% (AR-06), while TS and EAB increased from 2.752 MPa/6.241% to 3.941 MPa/10.235%, a 43% gain in tensile strength. AR-06 produced the largest inhibition zones against *Pseudomonas aeruginosa* and *Bacillus subtilis* among all tested formulations. In a food spoilage test, AR-06-sealed milk maintained a pH of 7.0 after 48 h at 25°C, compared with a pH drop to 6.0 in the unsealed control. Soil burial tests confirmed that all films are biodegradable, with AR-06 exhibiting the highest weight loss over four weeks. Taken together, these results identify AR-06 (0.5 g frankincense ethanolic extract per 50 g film solution) as the optimal formulation, balancing mechanical strength, moisture resistance, antimicrobial activity, and biodegradability for sustainable active food packaging applications. Unlike hydro-distillation-derived essential oil, the Soxhlet ethanolic method is expected to retain high-molecular-weight boswellic acid constituents, which may account for the strong interaction with the hydroxyl-rich PVA/starch matrix.

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Declaration of Competing Interest

There is no competing interest.

Data availability

Data will be available on request.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the author(s) used ChatGPT to improve the language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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