# *Tradescantia pallida* extract incorporated chitosan/ pullulan intelligent biodegradable films: An eco-friendly packaging to preserve the freshness of chicken

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**Table S1. Optimization table for base matrix CS/PU**

| CS (in g) | PU (in g) | Observation |
|---|---|---|
| **2.0** | **0.0** | **Non-peelable film** |
| **1.5** | **0.5** | **Good Film (With superior antimicrobial properties) ✓** |
| **1** | **1** | **Good film** |
| **0.5** | **1.5** | **Highly brittle film** |
| **0.0** | **2.0** | **Non-peelable film** |

**Table S1.1 Composition table of control CP and CPT intelligent biodegradable Films**

| Sample Code | Chitosan (g) | Pullulan (g) | Glycerol (V/V) (mL) | Tradescantia pallida Extract (mg) |
|---|---|---|---|---|
| **CP** | 1.5 | 0.5 | 10 | _ |
| **CPT-1** | 1.5 | 0.5 | 10 | 100 |
| **CPT-2** | 1.5 | 0.5 | 10 | 200 |

**Table S2. Zone of inhibition and CFU of control CP and CPT intelligent biodegradable films on various food bone pathogens**

| | Antimicrobial Activity ( Zone of Inhibition in mm) | | | |
|---|---|---|---|---|
| | ***B. subtilis*** | ***S. aureus*** | ***E. coli*** | ***C. albicans*** |
| • **CP** | 18 ± 0.17$^{c}$ | 14 ± 0.11$^{c}$ | 14 ± 0.17$^{a}$ | 13 ± 0.11$^{c}$ |
| • **CPT-1** | 23 ± 0.19$^{b}$ | 16 ± 0.19$^{a}$ | 17 ± 0.16$^{b}$ | 14 ± 0.13$^{a}$ |
| • **CPT-2** | 26 ± 0.24$^{a}$ | 17.5 ± 0.18$^{b}$ | 18.5 ± 0.15$^{c}$ | 15 ± 0.12$^{b}$ |

| Colony Forming Unit (CFU, $10^{-2}$ Dilution, Analyzed by Open CFU Software) | | | | |
|---|---|---|---|---|
| | **Control** | **CP** | **CPT-1** | **CPT-2** |
| • ***B. subtilis*** | 10632000 | 8520000 | 6220000 | 2576000 |
| • ***S. aureus*** | 6972000 | 4900000 | 2472000 | 1252000 |
| • ***E. coli*** | 8072000 | 5872000 | 3500000 | 2250000 |
| • ***C. albicans*** | 12476000 | 10632000 | 8424000 | 7050000 |

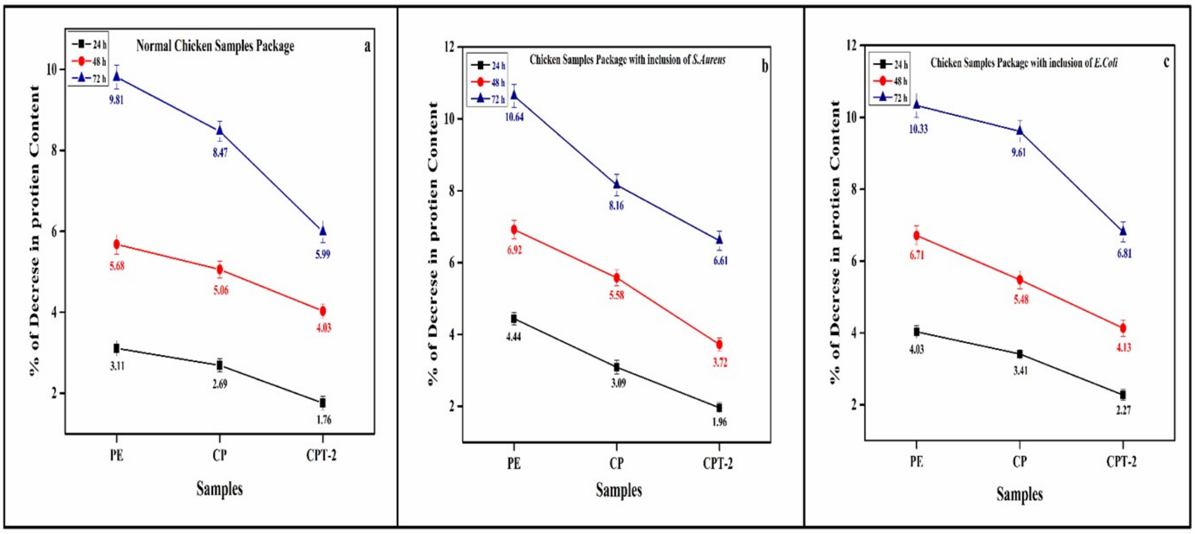
**Table S3 Total bacterial count (TBC) of normal and inclusion of pathogens (*S.aureus* and *E.Coli*) chicken meat in PE, control CP and CPT-2 intelligent biodegradable packaging**

| Total Bacterial Count (TBC): (Log of CFU/g of Chicken) | | | |
|---|---|---|---|
| | PE | CP | CPT-2 |
| 24 h | 6.55±0.0021[a] | 6.28±0.0016[c] | 5.51±0.0020[b] |
| 48 h | 8.62± 0.0015[c] | 8.25±0.0019[a] | 6.99±0.0017[b] |
| 72 h | 9.77±0.0017[b] | 9.63±0.0018[a] | 7.49±0.0016[c] |
| **Total Bacterial Count on the inclusion of *S.aureus* (Log of CFU/g of Chicken)** | | | |
| 24 h | 7.36±0.0014[b] | 7.19±0.0017[a] | 5.67±0.0015[c] |
| 48 h | 8.59±0.0011[c] | 8.37±0.0015[a] | 7.51±0.0013[b] |
| 72 h | 10.89±0.0022[a] | 10.78±0.0020[b] | 8.84±0.0018[c] |
| **Total Bacterial Count on the inclusion of *E.Coli* (Log of CFU/g of Chicken)** | | | |
| 24 h | 7.67±0.0011[c] | 6.48±0.0014[a] | 6.53±0.0012[b] |
| 48 h | 9.81±0.0015[c] | 9.66±0.0019[a] | 7.84±0.0017[b] |
| 72 h | 10.87±0.0021[b] | 10.80±0.0023[a] | 8.97±0.0020[c] |

**Table 4** Compression of tensile strength, antimicrobial and antioxidant properties of different intelligent compounds incorporated in the polymer blend films

| **Film Composition** | **Intelligent Agent** | **Tensile Strength (MPa)** | **Zone of Inhibition (in mm)** | | | | **Antioxidant activity (%)** | | **References** |
|---|---|---|---|---|---|---|---|---|---|
| | | | **S. aureus** | **B. subtilis** | **E. coli** | **C. albicans** | **DPPH Assay** | **ABTS Assay** | |
| **1Chitosan/ Fucoidan** | **Coleus grass** | 35.83 | 23.45 | -- | 25.69 | -- | 86.03 | 92.36 | [1] |
| **2Guar gum/ PVA and** | **hyacinth bean** | 12.67 | -- | -- | -- | -- | 82.74 | 94.61 | [2] |
| 3Cassava starch/PVA | **Aronia melanocarpa** | 23.07 | -- | -- | -- | -- | -- | -- | [3] |
| Chitosan/Methylcellulose | **Phyllanthus reticulatus** | 68.00 | 36.4 | | 35.8 | | 84.69 | -- | [4] |
| **5PVA/Chitosan** | **Sweet purple potato** | 30.8 | 12 | -- | -- | -- | -- | 91.00 | [5] |
| **6Chitosan/polycaprolactone nanofibrous** | **shikonin** | 7.46 | -- | -- | -- | -- | 88.57 | 62.74 | [6] |
| **7Chitosan/ Cellulose nanocrystals** | **blueberry** | 35.07 | -- | -- | -- | -- | 79.00 | -- | [7] |
| **8Pullulan/Chitin nanofibers** | **ATH** | 4.3 | 22.67 | | 22.83 | | 61.72 | -- | [8] |
| **Methylcellulose/Chitosan nanofiber** | **Barberry** | 40.47 | -- | -- | -- | -- | 85.12 | -- | [9] |
| **Chitosan/Pullulan** | ***Tradescantia pallida*** | 45.84 | 17.5 | 26 | 18.5 | 15 | 89.81 | 85.14 | **Present study** |

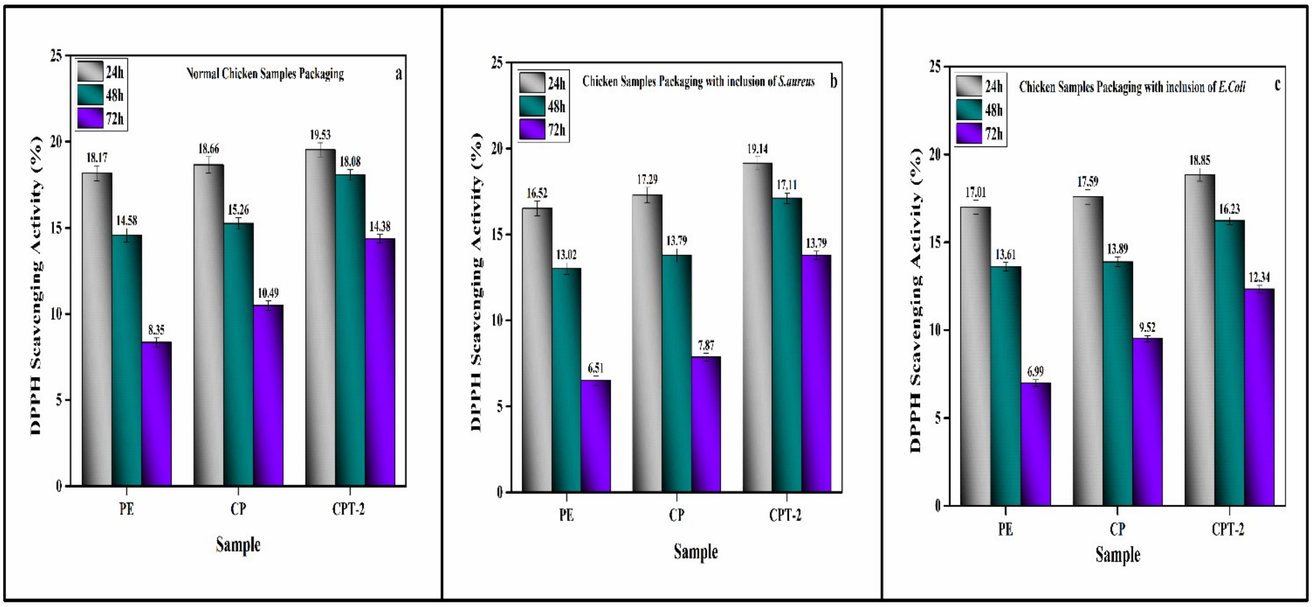
## S.6.Results and Discussions




### *S.6.1. Total Protein Content*

**Fig. S1 Estimation of % of decrease in protein content after preservation of Chicken meat in PE, control CP, and CPT-2 intelligent biodegradable films**

Chicken meat spoiling also impacts the protein content. However, compared to PE and CPT-2 intelligent biodegradable packaging, the percentage of protein content reduction was higher in plastics due to packed chicken in PE packaging being vulnerable to microbiological and oxidative attacks. On the other hand, because of the polyphenolic (anthocyanin) content of TP extract, CPT-2 exhibits intrinsically higher antibacterial and antioxidant properties. Therefore, as an active and intelligent packaging material for chicken meat, CPT intelligent biodegradable could be an alternative to traditional PE packaging.




***S.6.2. Antioxidant Capability of Chicken Meat After Incubation Period***

**Fig. S2 Estimation of antioxidant capacity of chicken meat in PE, control PC, and CPT-2 intelligent biodegradable films**

***S.6.3. Cost analysis and LCA of CPT-2 intelligent packaging film***

The intelligent biodegradable film formulation consists of 1.5 g of chitosan, 0.5 g of pullulan, and 0.2 g of *Tradescantia pallida* extract, totaling 2.2 g. To assess commercial feasibility, the composition was scaled to 1 kg of finished film, corresponding to approximately 681.82 g of chitosan, 227.27 g of pullulan, and 90.91 g of extract. Based on current Indian market rates (October 2025, ₹88 = USD 1), the raw material costs approximately were estimated using prices of ₹2,183/ kg for chitosan, ₹5,900/ kg for pullulan, and ₹1,000/ kg for the plant extract. Accordingly, the individual material costs per kilogram of film are ₹1,488.41 for chitosan, ₹1,340.91 for pullulan, and ₹90.91 for the extract, giving a total raw material cost of ₹2,920.23 / kg. Additional processing costs, including solvents and consumables (₹200 / kg), labor and quality control (20 % of raw material cost, i.e., ₹584 / kg), and equipment amortization (₹200 / kg), raise the total manufacturing cost to approximately ₹3,904.23 / kg or USD 44.37 / kg. On a laboratory scale, producing a 2.2 g film sample costs about ₹8.6 (USD 0.10). In comparison, commercial low-density polyethylene (LDPE) film, commonly priced around ₹160 / kg (USD 1.82 / kg), costs only ₹0.35 (USD 0.004) for a 2.2 g equivalent. Thus, the intelligent biodegradable film is nearly 24 times more expensive than LDPE, primarily due to the high costs of pullulan and chitosan.

**Table S4 Tentative Cost estimation CPT-2 Intelligent Packaging film**

| Parameter | Details / Calculation |
|---|---|
| **Material Composition (per 1 kg film)** | 681.82 g Chitosan + 227.27 g Pullulan + 90.91 g *T. pallida* extract |
| **Unit Price** | Chitosan – ₹2,183 /kg, Pullulan – ₹5,900 /kg, T. pallida extract – ₹1,000 /kg |
| **Raw Material Cost (per 1 kg film)** | Chitosan = 0.68182 kg × ₹2,183 = ₹1,488.41<br>Pullulan = 0.22727 kg × ₹5,900 = ₹1,340.91<br>T. pallida extract = 0.09091 kg × ₹1,000 = ₹90.91 |
| **Total Manufacturing Cost (1 kg film)** | Raw + Additions = INR 3,904 |

**Table S5 Comparison table of CPT-2 and PE packaging material**

| Film Type | Approx. Cost per kg (₹) | Approx. Cost per kg (USD) | Cost per 2.2 g sample (₹) | Cost per 2.2 g sample (USD) | Relative Cost Difference |
|---|---|---|---|---|---|
| **Biodegradable (CPT-2)** | 3,904 | 44.4 | 8.59 | 0.098 | Presently PE packaging ~24× cheaper |
| **LDPE (PE film)** | 160 | 1.82 | 0.35 | 0.004 | |

However, the Life Cycle Assessment (LCA) comparison between the CPT-2 intelligent biodegradable film and conventional polyethylene (PE) film highlights significant environmental and functional differences. The CPT-2 film is derived from renewable biomass sources, including chitosan (derived from chitin), pullulan (produced through microbial fermentation), and plant extracts, with an estimated energy consumption of approximately 46–60 MJ/kg. In contrast, PE film is petroleum-based and relies on energy-intensive extraction and polymerization processes that consume 70–90 MJ/kg. In terms of greenhouse gas emissions, CPT-2 film may generate about 1.0–1.7 kg $CO_2$ equivalent per kg, benefiting from renewable carbon cycling, whereas PE film produces 1.98–3.5 kg $CO_2$ equivalent per kg due to its dependence on fossil fuels. Regarding biodegradability and end-of-life behavior, CPT-2 film is fully biodegradable within 2–6 months under composting conditions, generating no microplastics and posing minimal risk to marine pollution, while PE film is non-biodegradable, persisting in the environment for 500–1,000 years and contributing significantly to microplastic pollution and aquatic toxicity. In terms of resource utilization, CPT-2 film requires agricultural land for feedstock cultivation but relies on renewable resources, with moderate water usage mainly for crop irrigation. PE film, although not dependent on agricultural land, depletes finite fossil fuel reserves and causes environmental degradation during extraction. Functionally, the CPT-2 film demonstrates superior performance by extending chicken shelf life to two days at room temperature due to its antimicrobial properties, with all components being FDA-approved for food safety. In contrast, PE film preserves chicken for only one day, lacks antimicrobial activity, and poses potential risks of chemical migration.

Although CPT-2 intelligent biodegradable films currently possess a technology readiness level (TRL) of 4–5 and with a cost barrier as compared to the substantial pollution from conventional plastic packaging, as evidenced by life cycle assessment (LCA) studies, underscores the importance of focusing future research on developing biodegradable polymer films for food packaging applications. Further investigations should focus on developing novel cost optimization strategies to enable these materials to effectively compete with fossil fuel-based plastic packaging, which poses significant environmental threats for the future.

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