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Evaluation of Antimicrobial Properties of PVA/*Leucaena leucocephala* Film to Preserve Beef Meat Quality

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ABSTRACT

Active food packaging refers to food packaging that are incorporated with components such as antimicrobial agents that would release or absorb substances into or from the packaged food. These interactions would maintain or prolong the shelf life of the food while maintaining their quality and safety. Polyvinyl alcohol (PVA) is one of the most well-known synthetic biodegradable polymers and possess an excellent mechanical property. In this research, an attempt was made by incorporating Leucaena leucocephala (1, 5, 10 and 15 % w/v) to the PVA film (85-99 % w/v) by using casting method to enhance the antimicrobial activity and the degradation rate of the film. Various tests such as Total Viable Count (TVC), Coliform Count (CC) and Total Chlorophyll Content (TCC) was carried out to determine the effectiveness of the antimicrobial properties of the film by using beef meat model. It was found that 15 % w/v of leaves incorporated with 85 % w/v PVA film was shown as the best antimicrobial film with the lowest microbial concentration of 5.5 x10^7 CFU/ml and 4 X 10^6 CFU/ml for TVC and CC, respectively compared to the other film formulations.

Keywords: Antimicrobial, beef meat model, blend film

1. INTRODUCTION

Plastic-based packaging which is often petroleum-derived and non-biodegradable is known to contribute significantly to environmental pollution because it does not degrade easily and accumulates as waste. Therefore, growing concern over non-biodegradable plastic waste and its environmental impact has led to an increasing demand for biodegradable plastics. The global market for biodegradable polymers is expected to expand due to their rising popularity across a wide range of end-user industries.

Biodegradable packaging materials are increasingly studied as sustainable alternatives. Biopolymers and polymer blends such as polyvinyl alcohol (PVA) offer an eco-friendly substitute, because they can degrade under appropriate conditions and reduce reliance on fossil-fuel plastics (Shah et. al., 2024). PVA is especially attractive for food packaging applications due to its transparency, film-forming ability, water-solubility (which aids biodegradation), and compatibility with additives (Hussain et. al., 2023). By incorporating natural plant-derived bioactive materials into PVA-based films, it is possible to develop "active packaging" that not only protects food physically, but also inhibits microbial growth and thereby extends food's shelf life.

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The approach of combining PVA with locally available plant-based resources such as *Leucaena leucocephala* (also known in Malaysia as "Petai Belalang") appears promising. The natural compounds present in *L. leucocephala* (e.g., phenolics, tannins, proteins, carbohydrates) could contribute antimicrobial, barrier, or structural functions, potentially producing biodegradable active packaging suitable for food application (Elbanoby et. al., 2024). To date, there have been no research yet about PVA incorporated with *L. leucocephala*.

This study aims to develop biodegradable films by incorporating different ratios of *Leucaena leucocephala* seeds and leaves into PVA, in order to determine the optimal formulation based on Total Viable Count (TVC), Coliform Counts (CC), and TCC analyses. The need for environmentally friendly food packaging is increasingly critical, as such materials can help reduce waste and contribute positively to environmental sustainability.

2. MATERIALS AND METHODS

2.1 Chemicals

Poly (vinyl alcohol) (99-100%) were purchased from Sigma Aldrich. Acetone was purchased from HmBG.

2.2 Plant materials

The *L. leucocephala* leaves and seeds were collected from an abandoned field in Wang Ulu, Kangar, Perlis. The freshly obtained leaves and seeds were then rinsed thoroughly with distilled water. The leaves and seeds were dried in an oven (FD 115, Binder, United States) at 60 °C for 24 hours. Then it was ground using a grinder (AS 200, RT Precision Tech, Germany) and sieved to 150 µm particle size using a siever (AS 200, RT PrecisionTech, Germany).

2.3 Film preparation

The PVA/*L. leucocephala* leaves and seeds film were prepared by casting method. Film solution was prepared by dissolving 8.5 g of PVA in 1 L sterile water at 85 °C for 1 hour until the PVA was totally dissolved using a hot plate stirrer (100 rpm). Then, 15 g of *L. leucocephala* leaves powder was then dissolved in 1 L distilled water at 40 °C for 1 hour using a hot plate stirrer (100 rpm). Thereafter, the leaves solution was added to PVA solution at 40 °C for 30 minutes. The final suspension was casted onto a glass petri dish with a constant volume of 25 ml to ensure the consistency of the film thickness and were left to dry in the oven at 60 °C for 24 hours and peeled off using forceps from the petri dish to obtain a dried film. The PVA/*L. leucocephala* seeds films were prepared using the same method with different concentration as shown in Table 1.

2.4 Preparation of Beef Meat Model

Fresh beef was obtained from market located at Kangar, Perlis, and used as a model sample for this study. The beef meat model was cut into cubes of 2cm x 2cm x 2cm. The fresh beef meat model was coated with the prepared films and placed in the refrigerator at 10 °C for 9 days.

2.5 Preparation of Nutrient Agar and Red Bile Agar

First, 28 g of nutrient agar powder (Merck, Malaysia) was suspended into 1 L of distilled water. The solutions were heated using a hot plate. After that, the solutions were autoclaved at 121 °C. The solution was allowed to cool down first. Then, the nutrient agar was poured into each plate, and the plates were left on a sterile surface until the agar solidified. The same procedure was used to prepare red bile agar (Merck, Malaysia) by dissolving 39.5 g of agar powder in 1 L of distilled water.

Table 1: Formulation of PVA / *L. leucocephala* films

Formulation	Amount of PVA (% w/v)	Amount of Leaves (% w/v)	Amount of Seeds, (% w/v)
1	100	0	0
2	85	15	0
3	85	0	15
4	90	10	0
5	90	0	10
6	95	5	0
7	95	0	5
8	99	1	0
9	99	0	1

2.6 Antimicrobial Activity

2.6.1 Total Viable Count (TVC)

The samples were cultivated on nutrient agar. Then, the nutrient agar was sealed and incubated at 37 °C for 24 hours. Thereafter, the inocula were prepared by washing the growing culture with 10 ml of sterilized water and rubbed with hockey stick. Then, the optical density of suspensions were measured at absorbance of 600 nm by using a UV Spectroscopy (GENESYS 20, Germany). The experiments were performed over 3 days.

2.6.2 Coliform Count (CC)

The samples were cultivated on violet red bile agar and incubated at 37 °C for 24 h. The inoculum was then prepared by rinsing the resulting colonies with 10 mL of sterile water. The cell suspensions were homogenized using a sterile hockey stick and their absorbance was measured at 600 nm using a UV–Vis spectrophotometer. The experiments were conducted over three consecutive days, during which samples were collected for coliform count analysis to determine the bacterial concentration.

2.6.3 Antioxidant Activity

The antioxidant properties of the films were carried out by measuring the total chlorophyll content in the film. The film prepared were mixed with 50 % of acetone in dark conditions. All the samples being analyzed were read at 646 nm, then read again after resetting the wavelength to 663 nm by using UV spectroscopy. Calculations for total chlorophyll concentrations were made after the absorbance were read at both wavelengths using equation (1).

$$TCC = 17.34\, A_{646} + 7.18\, A_{663} \tag{1}$$

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity Test

3.1.1 Total Viable Count (TVC)

A high total viable count indicates a high concentration of microorganisms in the food product. Table 2 shows the result of the viable cell concentration towards the antimicrobial properties of *L. leucocephala* leaves and seeds blend films after 3 days of storage.

Table 2: Viable cell concentration towards the antimicrobial properties of *L. leucocephala* leaves and seeds blend films

Amount of leaves/seeds (% w/v)	Viable Cell Concentration (CFU/ml)	
	PVA/*L. leucocephala* Leaves	PVA/*L. leucocephala* Seeds
0	$1.52x10^9$	$1.52x10^9$
1	$1.09x10^9$	$1.40x10^9$
5	$5.63x10^8$	$6.34x10^8$
10	$2.99x10^8$	$4.33x10^8$
15	$5.50x10^7$	$3.54x10^8$

The viable cell concentration of the *PVA/L. leucocephala* leaves and seeds blend films were highly influenced by the *L. leucocephala* leaves and seeds content. For PVA/L. *leucocephala* leaves blend films, it can be seen that 15.0 % w/v PVA/L. *leucocephala* leaves blend films showed the highest antimicrobial activity with the lowest viable cell concentration of $5.5x10^7$ CFU/ml, while the highest viable cell concentration can be found in 1.0 % w/v PVA/L. *leucocephala* leaves with $1.09x0^9$ CFU/ml after 3 days of storage at 10 °C. For PVA/L. *leucocephala* seeds blend films, 15.0 % w/v PVA/L. *leucocephala* seeds blend films showed the highest antimicrobial activity with the lowest viable cell concentration of $3.54x10^8$ CFU/ml, while the highest viable cell concentration can be found in 1.0 % w/v PVA/L. *leucocephala* seeds with $1.4x10^9$ CFU/ml after 3 days of storage at 10 °C. Based on Figures 1 and 2, the results showed a consistency of increasing growth of viable cell concentration for both PVA/L. *leucocephala* leaves and seeds blend films. The 15.0 % w/v PVA/L. *leucocephala* leaves blend films remained highest in antimicrobial activity compared to the 15.0 % w/v PVA/L. *leucocephala* seeds blend films after 9 days of storage.

Generally, *L. leucocephala* leaves contain good antimicrobial and antioxidant properties due to the presence of tannins, saponins, flavonoids, phlobtanins, steroids, and glycosides (Mohammed et al., 2015). The presence of tannins are capable as antioxidant and antimicrobial in the *L. leucocephala* leaves as it precipitates the protein and inhibit the enzyme reverse transcriptase, thus there is no microbial cell formation. Moreover, tannins are the metabolite compounds that played an important function in inhibiting microbial activity. Meanwhile, the seeds of Leucaena do not exhibit strong antimicrobial activity, which limits their ability to suppress or inhibit microbial growth.

For the PVA/*L. leucocephala* leaf-blend films, the greatest antimicrobial activity against *E. coli* was observed in the 15.0% (w/v) formulation, which exhibited the lowest bacterial count of 4.0×10^6 CFU/mL after 3 days of storage at 10 °C. In contrast, the highest bacterial growth **(**8.60×10^7 CFU/mL) occurred in the 1.0% (w/v) leaf-blend films.

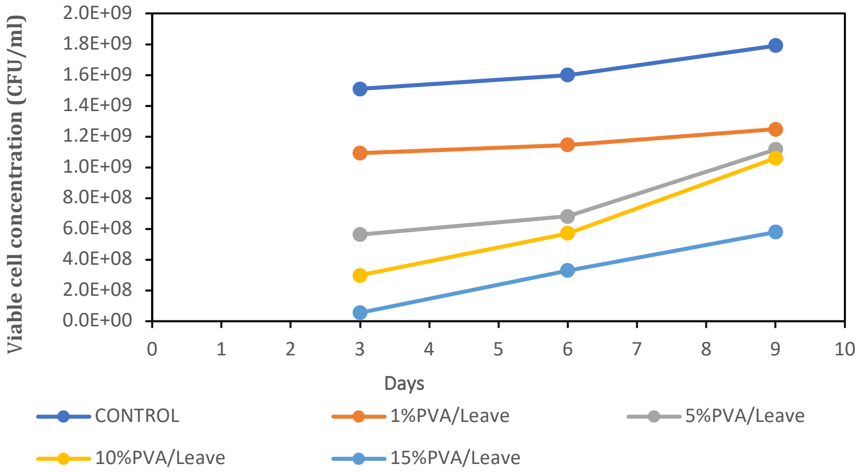

Figure1: Growth of viable cell concentration on PVA/*L. leucocephala* leaves blend films during storage of 9 days at 10 °C.

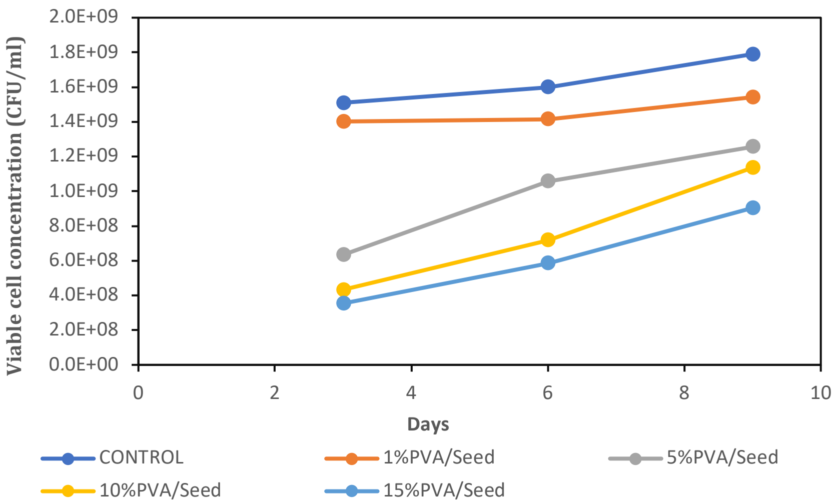

Figure 2: Growth of viable cell concentration on PVA/*L. leucocephala* seeds blend films during storage of 9 days at 10 °C.

For the PVA/*L. leucocephala* seed-blend films, the strongest antimicrobial effect was also recorded in the 15.0% (w/v) formulation, with the lowest bacterial count of 1.02 × 10^8 CFU/mL after 3 days at 10 °C. The highest bacterial load (1.29 × 10^9 CFU/mL) was detected in the 1.0% (w/v) seed-blend films.

3.1.2 Coliform Count (CC)

Total coliforms were used as indicators to measure the degree of contamination and sanitary quality of food product because testing for all known pathogens is a complicated and expensive process. It measures the concentration of total coliform bacteria associated with the possible presence of disease-causing organisms. Gram-negative bacteria such as *E.coli* were used to study the antimicrobial activity of the PVA/*L. leucocephala* leaves and seeds blend films as shown in Table 3.

Table 3: *E.coli* concentration towards the antimicrobial properties of *L. leucocephala* leaves and seeds blend films

Amount of leaves/seeds (% w/v)	*E.coli* Concentration (CFU/ml)	
	PVA/*L. leucocephala* Leaves	PVA/*L. leucocephala* Seeds
0	1.41x0^{9}	1.41x10^{9}
1	8.60x10^{7}	1.29x10^{9}
5	4.30x10^{7}	3.44x10^{8}
10	1.60x10^{7}	2.36x10^{8}
15	4.000x10^{6}	1.02x10^{8}

Based on Figures 3 and 4, both the PVA/*L. leucocephala* leaf- and seed-blend films showed a consistent increase in *E. coli* concentration over the 9-day storage period. However, the 15.0% (w/v) PVA/*L. leucocephala* leaf-blend films continued to exhibit the strongest antimicrobial activity, demonstrating lower *E. coli* growth compared to the corresponding 15.0% (w/v) seed-blend films throughout the storage duration.

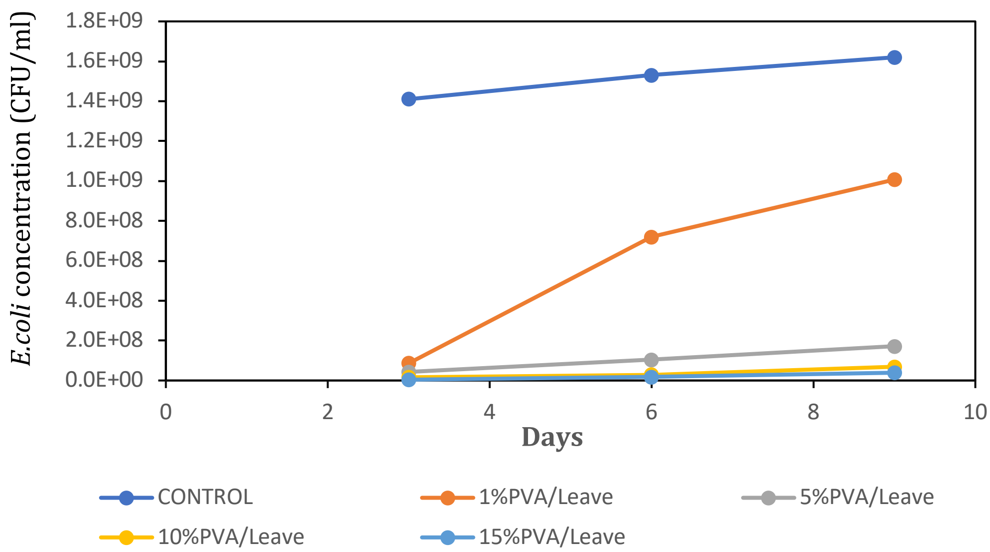

Figure 3: Growth of *E.coli* concentration on PVA/*L. leucocephala* leaves blend films during storage of 9 days at 10 °C

Therefore, the 15% (w/v) PVA/*L. leucocephala* leaf-blend films exhibited the highest antimicrobial activity, in contrast to the 1% (w/v) PVA/*L. leucocephala* seed-blend films, which showed the lowest activity. Both the leaf- and seed-based PVA blend films demonstrated greater susceptibility toward *E. coli* compared to pure PVA, indicating that the incorporation of *L. leucocephala* as an active compound enhanced the antimicrobial properties of the films. Overall, the results identified 15% (w/v) *L. leucocephala* leaf content as the most effective concentration for producing antimicrobial PVA blend films. High phenolic compound in the leaves compared to the seed might be contributing to the high antimicrobial activity. These results are consistent with the study conducted by Andrade et al. (2022), who discovered that the growth of *E. coli* and coliforms in beef kept at low temperatures was inhibited by active multilayer films containing

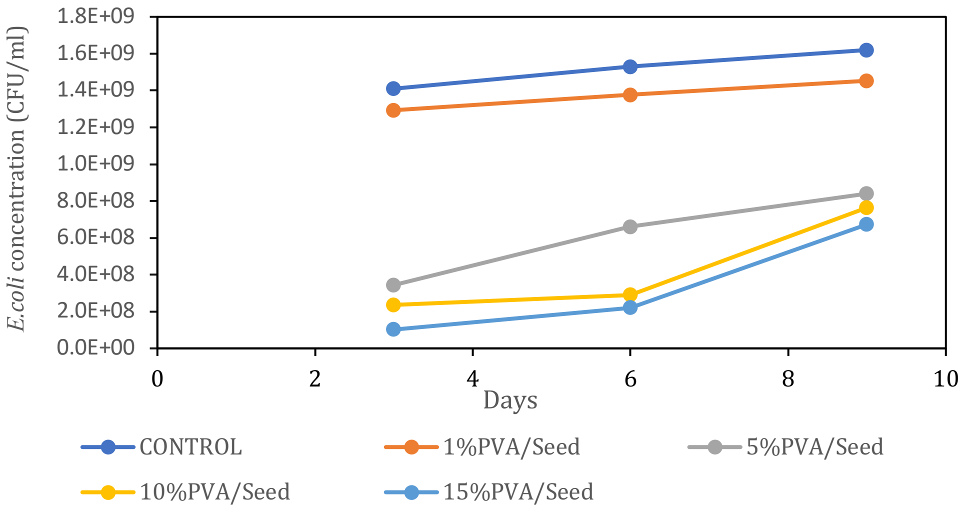

Figure 4: Growth of *E.coli* concentration on PVA/L. *leucocephala* seeds blend films during storage of 9 days at 10 °C

natural phenolic compounds. In their study, meat samples packaged with active films showed much slower increase in coliform counts compared to the control. This supports the idea that adding leaves/seeds into packaging materials can be a practical way to control harmful bacteria and extend the shelf life of fresh meat products.

3.2 Antioxidant Activity Test

3.2.1 Total Chlorophyll Content

Antioxidants are compounds that are capable of protecting the cells in a substance from damage caused by free radicals. Free radicals are an uncharged molecules that are made during normal cell metabolism where they can react and form chain reactions with other molecules. The molecules which they react with are damaged and turn into free radicals. Thus, antioxidants played important functions by reducing the oxygen concentration, breaking down the species that initiate peroxidation and free radical chain reactions (Badarinath et al., 2010). Antioxidants react by contributing free radicals a hydrogen atom during oxidation and also transform themselves into radical form. Nevertheless, these radical intermediates are stable because of resonance delocalization of the extra electron within the aromatic ring and subsequent formation of stable quinones (Nawar, 1996). This potently delays oxidation of lipid, thus preserving the meat products' quality and shelf-life. Phenolics found in natural antioxidants possess vigorous hydrogen atom donating activity. Phenolic acids, phenolic diterpenes, flavonoids, and volatile oils are the major antioxidative phenolics compounds. Both phenolic acids and flavonoids reacts by scavenging free radicals (Brewer, 2011). Table 4 shows the result of the total chlorophyll content of *L. leucocephala* leaves and seeds blend films.

Table 4: Total chlorophyll content of PVA/L. *leucocephala* leaves and PVA/L. *leucocephala* seeds after storage of 6 days.

Amount of leaves/seeds (% w/v)	**PVA/L. *leucocephala* Leaves**		**PVA/L. *leucocephala* Seeds**	
	Day 3	**Day 6**	**Day 3**	**Day 6**
1	1.3994	0.9981	2.5099	0.7995
5	0.6955	0.2093	1.0603	0.3809
10	0.6363	0.1674	0.7141	0.3032
15	0.3175	0.0909	0.4503	0.2613

Based on Table 4, antioxidant trap free radical at relatively high concentrations only. Among the different concentration of PVA/L. *leucocephala* blend films, 15 % w/v PVA/*L. leucocephala* leaves and seeds blend films had significantly lower total chlorophyll content after 6 days of storage. The higher chlorophyll (or plant-pigment) content in the 15% w/v PVA/*L. leucocephala* blend film likely enhanced the film's antioxidant and antimicrobial potential, thus slowing oxidation and microbial proliferation in the wrapped meat. In contrast, the 1 % w/v film did not contain enough bioactive pigment to scavenge free radicals generated on the meat surface, resulting in oxidative stress and increased protein and lipid degradation. Therefore, oxidative stress states can occur once the amount of free radicals exceeded the amount of antioxidants. Oxidative stress is a state where there is an interruption balance between the free radicals and antioxidant defences. Thus, oxidative damage can occur to the proteins and lipids of the meat products (Moura-Alves et. al., 2023). Eventually, the antioxidant progressively depleted during the storage of 6 days. This can be explained by the radicals in the meat sample escaped with the antioxidants and lead to increased hydroperoxides concentration when the antioxidant concentration finally fell below the threshold level. The radical's concentration further rises, as the hydroperoxides concentration accumulated which led the remaining antioxidant compounds are completely used up. The antioxidants in the film progressively depleted as they scavenge free radicals generated in the meat. The total chlorophyll content decreased with the higher concentration of *L. leucocephala* leaves and seeds. The antioxidant capacity of chlorophyll in *L. leucocephala* leaves and seeds could be due to the presence of natural antioxidants. *L. leucocephala* leaves and seeds contain high amounts of bioactive phytochemicals, phenolic compounds and nutrients (Ojo & Fagade, 2002). The phenolic compounds possess high antioxidant activity where it acts by adsorbing and neutralizing the free radicals and decomposing peroxides.

3.3 Beef Meat Model

The physical properties of the meat samples wrapped in different films were observed over 9 days of storage. As shown in Figure 5, the film with 1, 5 and 10 % w/v except for leaves appeared to be brownish in colour which indicates the sign of deterioration of meat. These visual signs of spoilage corresponded with higher TVC and CC values recorded for these films, indicating rapid bacterial growth. In contrast, meat sample wrapped with 15 % w/v PVA/L. *leucocephala* seed and 10, 15 % w/v PVA/*L. leucocephala* leaves retained a red color, suggesting improved preservation and lower microbial activity.

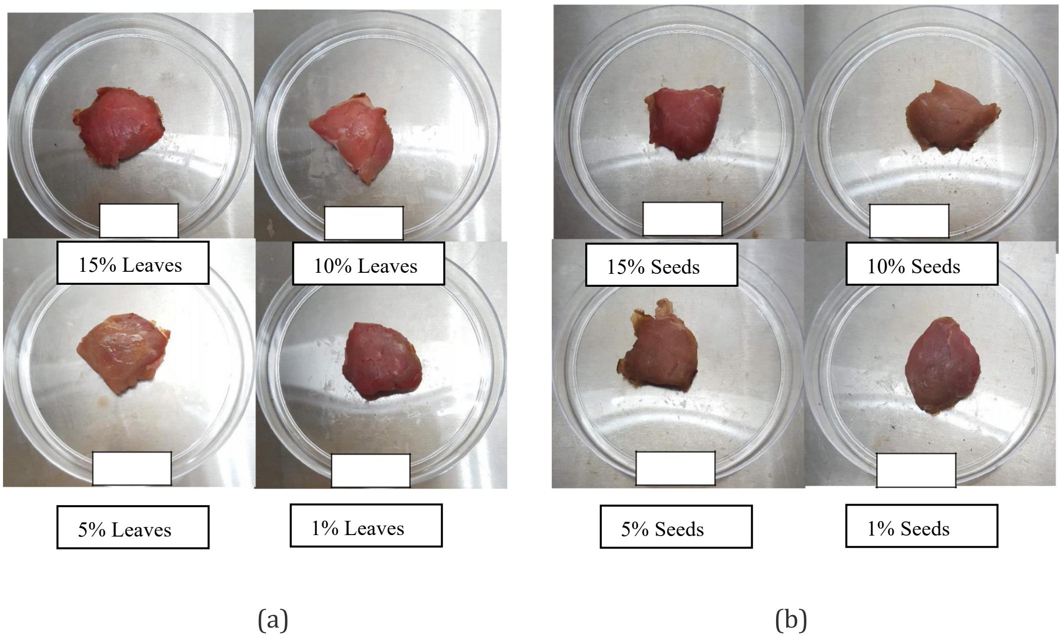

Figure 5: Condition of Beef Meat Model wrapped with PVA/*L. leucocephala* (a) leaves, and (b) seeds blend film after 9 days storage.

4. CONCLUSION

This study successfully developed an antimicrobial film based on a blend of PVA and *L. leucocephala* leaves/seeds for potential use in food packaging applications. The incorporation of *L. leucocephala* provides active compounds which showed antioxidant and antimicrobial activities. Films with 15 % w/v leave content effectively slowed the growth of total viable bacteria (TVC) and coliform bacteria (CC), helping preserve the red colour and freshness of the meat model system during chilled storage.

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