Development and Characterization of Antimicrobial Film from *Clinacanthus nutans* and Polyvinyl Alcohol (PVA) Mixture for Food Packaging

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Abstract

Clinacanthus nutans is a beneficial plant with antimicrobial, antidiarrheal, antiviral, antioxidant, antiinflammatory, and antidiabetic properties. This work focused on the development and characterization of antimicrobial film from blends of *C. nutans* and PVA for food packaging. The films were characterized in terms of morphology, physical and mechanical properties. Antimicrobial activities of the film blends were evaluated using the meat model to mimic packaging for meat. Principal component analysis (PCA) was carried out to discriminate the films and it was found that the PVA 99-100-*C. nutans* mixture results in the best performing film having similar antimicrobial performance with streptomycin blend films. The PVA 99-100-*C. nutans* blend film was further characterized using the universal testing machine, scanning electron microscope (SEM), and Fourier-transform infrared spectroscopy (FTIR). The *C. nutans* mixture blend film was found to have better mechanical properties than streptomycin blend film with its tensile strength of 0.8 and 0.4 MPa respectively. SEM images revealed that the surface of streptomycin blend film was rough and contains crystalline structures while the surface of the *C. nutans* mixture blend film contains lignin structures that help make the films more flexible. FTIR analysis reveals the presence of hydroxyl groups which also increases the tensile strength of the film. Based on the findings, blend film of *C. nutans* flour and PVA have a huge potential to be explored and commercialized as food packing and can be considered as new revolution in food industry.

Keywords: antimicrobial activity, Clinachanthus nutans, poly(vinyl alcohol), food packaging, antimicrobial film

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Introduction

In recent years, antimicrobials have gained a lot of attention because of their potential as antibiotics for many diseases. An antimicrobial is defined as a substance that can destroy and inhibit the growth of microorganisms. The use of biofilms with antimicrobial peptides against the growth of bacteria in medical devices have been tested [1]. The films can be further improved by adding active agents so it can be used to protect foodstuff from contamination.

Plant extracts typically have strong antioxidant properties due to the high concentration of phenolic compounds it possesses [2]. *Clinacanthus nutans* extracts also contain high concentrations of phenolic compounds and an added avantage of having good film forming properties. *C. nutans* contains flavonoids, stigmasterol, β-sitosterol, lupeol, botulin, C-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin, 7-O-β-glucopyranoside, orientin, isoorientin, cerebrosides, monoacylmonogalactosylglycerol, and sulfur-containing glucosides [3].

C. nutans consist of 73.27% carbohydrates. The carbohydrate content is mostly made up of starch and cellulose. *C. nutans* is used in industry to produce biodegradable films to partially or entirely replace plastic polymers for food packaging because of its low cost, renewability, and good mechanical properties [4]. Poly (vinyl) alcohol (PVA) is a type of synthetic polymer which has a crystalline structure and is soluble in water. It is produced by polymerising vinyl acetate through hydrolysis. PVA is also interesting as it can be easily decomposed by microorganisms. This polymer finds use in industrial, commercial, medical, and food sectors to produce an assortment of products [5]. One such use is for food packaging because of its favourable characteristics of being odourless, tasteless, and colourless [6]. It is also non-toxic and so it is used in pharmaceutical industries as enteric coatings for medication [6-7].

Antimicrobial film is a film with antimicrobial properties that follows the concept of active packaging to reduce or stop the growth of microorganisms on food. Many studies have shown that antimicrobial films are very efficient in reducing the levels of microorganisms such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium, Salmonella enteritidis, Yeusinia enterolitica,* and *Salmonella aureus* in food.

There are many types of antimicrobial agents that are commonly incorporated into food packaging. They include organic acid, enzymes, bacteriocins, polysaccharides, and essential oils. These films are biodegradable, safe, non-toxic, low cost, and efficient. Phenolic compounds in *C. nutans* such as flavonoids and tannins also have antimicrobial and anti-diarrheal properties [8]. Thus, *C. nutans* is a potential precursor material to use in biodegradable film production. This project develops film from blends of *C. nutans* and PVA and evaluates their effectiveness against microbial activity.

Materials and Methods

Chemicals

Poly (vinyl alcohol) (99-100%, 95%, 88% & 75% hydrolysis degree), sodium chloride, disodium hydrogen phosphate (anhydrous), and potassium dihydrogen phosphate were purchased

from Acros Organics. Violet Red Bile (VRB) agar powder and Plate Count (PCA) agar powder were purchased from Merck, Malaysia.

Materials

C. nutans was supplied by farmers from Kampung Wang Tepus, Jitra, Kedah. The plant was washed under running tap water and then cut and divided into three groups; stems, leaves, and mixture of stems and leaves. They were then dried in an oven (Binder, Malaysia) at 50 ˚C. The leaves take about 1 day to dry completely while the stems and mixture takes about 3 days to dry completely. The dried samples were ground into powder using a mechanical grinder (Mill Powder Tech, Taiwan) and sieved at 63 µm to produce a fine flour (Mahmud, 2010). For the microbiological study, fresh beef was purchased from a local market and cut into 2x2x2 cm cubes using a scalpel [9].

Film preparation

C. nutans flour/PVA blended films were produced using the casting technique. The film forming solutions were prepared by mixing *C. nutans* solution and PVA solution to specific ratios.

C. nutans solution

C. nutans (4 g) was mixed with distilled water (100 ml) to prepare *C. nutans* solution. The mixture was stirred and heated at 75 ˚C for at least 1 h using a magnetic stirrer. Then, starch gelatinization was accomplished by increasing the temperature to 85 ˚C, adding glycerol (15 ml/ 100 ml solution) and then stirring for at least 1 h [10].

PVA solution

PVA (4 g) and distilled water (100 ml) were mixed to prepare 10 to 100% (w/w) PVA solutions. The mixture was stirred and heated to 85 to 200 ˚C depending on the degree of hydrolysis of the PVA used, until all of the PVA was completely dissolved [10].

C. nutans/ PVA solution

The *C. nutans* and PVA solutions were mixed together to prepare mixture of the two solutions. The mixture was heated to 85 ˚C for 1 h with constant stirring carefully to avoid frothing. Water was added to the mixture to maintain their volume because heat will cause evaporate of the water [10]. Two control films were also prepared, one contains antibiotics and the other one without any antimicrobials or antibiotics.

Casting

Mixtures of *C. nutans* and PVA solutions were pipetted onto petri dishes. Air bubbles were removed using a flame and the solutions were dried for 24-72 h at 55 ˚C to form the films. Complete drying was avoided as some degree of moisture was required for the films to remain

flexible and not to crack. The films were then removed from the petri dish and placed in a desiccator to avoid moisture exchange [10].

Antimicrobial Properties (MEAT MODEL)

The antimicrobial activity of the blend films was tested using beef meat model. The meat was cut into 2 x 2 x 2 cm cubes using a scalpel. The surface of the meat samples was coated with stand-alone films. Triplicates of non-coated and coated samples were placed in covered petri dishes. The petri dishes were then put into another container and stored at 18 ˚C. For the microbiological analyses, each sample was obtained aseptically and immersed in 50 ml of sterile buffered peptone water for 2 min. The buffered peptone water was used to leech out any microorganisms. The total viable and coliform microorganism count were determined at day 0 to day 2. Agar plates incubated at 30˚C for 24 h were used to determine the viable count while Violet Red Bile Agar plates incubated at 30 ˚C for 48 h were used to determine the coliform count [9].

Total Chlorophyll Content

Total chlorophyll content was used as a measure for colour of the pure and blend films. In a dark room, acetone was added to the films to a 1:1 ratio. Then, the films were incubated for about 30 min. The absorbance of the mixture at 646 and 663 nm was measured using a UV-VIS spectrophotometer. The total chlorophyll content, chlorophyll a and chlorophyll b were calculated using Equation 1.

$$\text{Total chlorophyll content (TCC)} = 17.34A_{646} + 7.18A_{663} \quad \text{(Eq. 1)}$$

where A_{646} and A_{663} were the absorbance of samples at wavelength 646 and 663 nm respectively.

Statistical Analysis

Statistical analysis of the data was carried out using Tukey's Honest Significant Difference test and Principal Component Analysis. The data was analysed using Minitab® 17 Minitab Inc., Australia.

Characterization of the Films

Film Thickness

A calliper was used to measure the thickness of the film to the nearest 0.1 [9].

Tensile Properties

The true stress-Hencky strain curves for films were obtained using a universal testing machine (Hounsfield Ltd, United Kingdom). Tensile strength (TS) and elongation at break of the films were evaluated from these curves. The samples were cut into rectangles (10x1 cm). The equilibrated film samples were mounted onto the film extension grips and stretched at 10 mm min^{-1} until breakage. The measurements were carried out at room temperature [9].

Surface Morphology and Cross Sections

The surface morphologies and cross-sections of the films were determined using a scanning electron microscope (SEM) (Supra, Germany). The samples were first sputter-coated with platinum to improve their conductivity [9].

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy (Perkin Elmer, United States) was used to analyse the chain interactions in the blend films. The FTIR spectrum for each sample was recorded in the wavenumber range from 400 to 4000 cm^{-1}. The analyses were performed at room temperature [9].

Results and Discussion

Development of PVA films and Selection of the Films based on its Peelability

In this study, four types of PVA with different degrees of hydrolysis (99-100, 95, 88 and 75%) were composited with *C. nutans* flour and used to develop films with anti-microbial properties. A good film formation was when the produced film can be easily peeled from its support and has even thickness. All films produced except for PVA 75% show good film formation (Figure 1 (M)-(P)). Pure and blend films of PVA 75% (Figure 1 (M)–(P)) cannot be peeled off smoothly and cracks easily even after drying for 120 h. Table 1 shows the molecular weight of PVA 99-100%, PVA 95%, PVA 88%, and PVA 75%. The poor film-forming ability and 'watery' behaviour can be attributed to the low molecular weight of PVA 75% [10]. Therefore, films made using PVA 75% were not studied further.

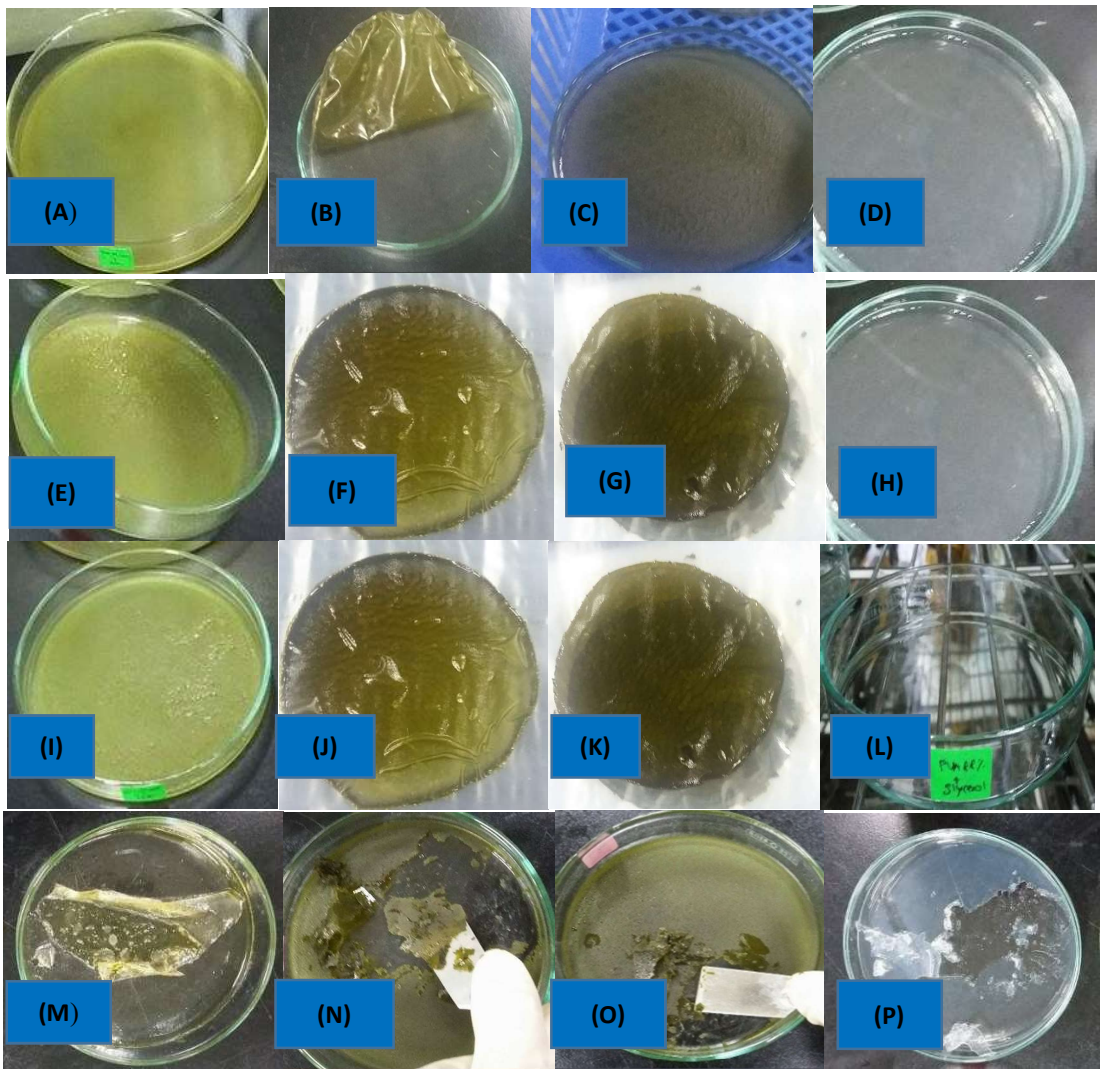

Figure 1: Different types of pure and blend films of PVA produced: (A) Blend film of PVA 99-100 – *C. nutans* stems (B) Blend film of PVA 99-100 – *C. nutans* mixture (C) Blend film of PVA 99-100 – *C. nutans* leaves (D) Pure film of PVA 99-100 (E) Blend film of PVA 95 – *C. nutans* stems (F) Blend film of PVA 95 – *C. nutans* mixture (G) Blend film of PVA 95 – *C. nutans* leaves (H) Pure film of PVA 95 (I) Blend film of PVA 88 – *C. nutans* stems (J) Blend film of PVA 88 – *C. nutans* mixture (K) Blend film of PVA 88 – *C. nutans* leaves (L) Pure film of PVA 88 (M) Blend film of PVA 75 – streptomycin (N) Blend film of PVA 75 – *C. nutans* mixture (O) Blend film of PVA 75 – *C. nutans* leaves(P) Pure film of PVA 75

Table 1: Characteristics of the blend films produced.

PVA types	Molecular weight
99-100%	124000
95%	95000
88%	88000
75%	2000

Antimicrobial Activities

Antimicrobial activity for food packaging is important to suppress activity of harmful microorganisms therefore increasing the shelf-life of products like meat [11]. In order to simulate food packaging of meat, the cubic fresh meat model was used to evaluate the antimicrobial activity of all films except the pure and blend films of PVA 75%.

Pure and blend films of PVA 88% were found to become sticky, adhering to the meat cube and therefore rendering it unusable (Figure 2). The low molecular weight and high degree of hydrolysis of PVA 88% causes the formed film to have high solubility and low water resistance, making it unsuitable as packaging for meat [5, 12]. Thus, these films were not studied further.

PVA 99-100 and PVA 95% films show good potential as packaging for meat as they remained stable even when the meat was moist. Both films have great water resistance proportional to the degree of hydrolysis and molecular weight which result in the low solubility of the films.

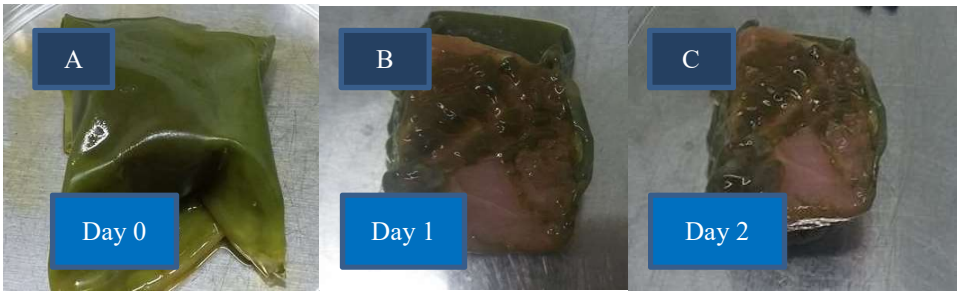

Figure 2: Meat model wrapped with blend film of PVA 88 – *C.nutans* mixture

Total Viable Count

Total viable count (TVC) is a method to measure the number of viable microorganism cells in a sample. This technique is sensitive and has the advantage of only counting living bacteria. All visible colonies are calculated and expressed as colony forming units (CFU).

Figure 3 shows the number of observed viable cells originating from pure and blend films of PVA 99-100%. The results show that blend films of PVA 99-100 – streptomycin (control films) have a lower initial CFU as it contains antibiotics which helps suppress the growth of microorganisms. However, the number of viable cells was found to increase over time. The blend films of PVA 99-100 – *C. nutans* stems, PVA 99-100 – *C. nutans* leaves, and pure PVA were found not significantly different in terms of number of viable cells after two days of incubation. The CFU for the PVA 99-100 – *C. nutans* mixture blend film was approximately equal to that for the control film initially, but was found to have reduced the most after incubation for one and two days. This may be due to the synergistic antimicrobial effect of tannins and flavonoid compounds present in *C. nutans* stems and leaves [13]. The tannins and flavonoids compounds in *C. nutans* stems or *C. nutans* leaves alone may not be as effective in suppressing microbial activity [8].

Figure 4 also shows that the blend film of PVA 95–streptomycin has the lowest CFU compared to the other films. After day 1, the CFU for PVA alone was the lowest compared to the negative control and blend films of *C. nutans* mixture, leaves, and stems. However, for the pure

PVA, the negative control and blend films of C. *nutans* mixture, leaves and stems were not significantly different in terms of CFU after two days of incubation.

PVA 99-100 – streptomycin blend film was found to be the best performing film even though there was an increment in colony number from day 1 to 2.

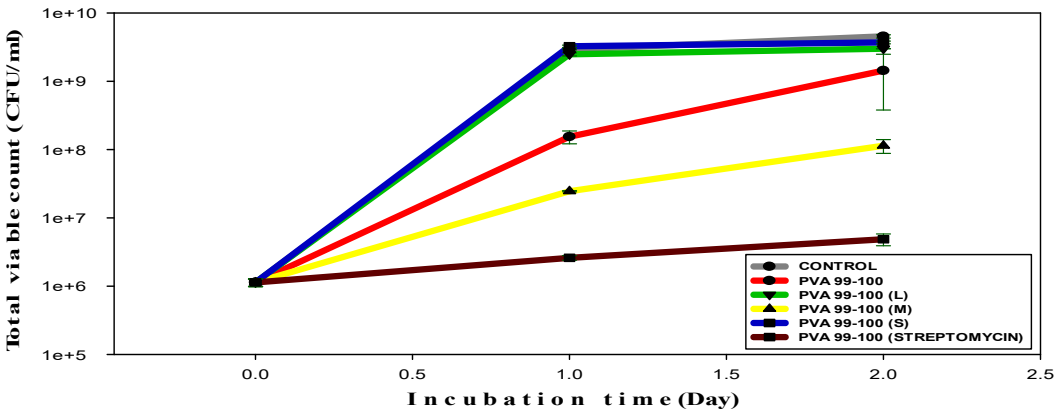

Figure 3: Total viable count of pure and blend films of PVA 99-100%. Each point is the mean ± SE (N = 3)

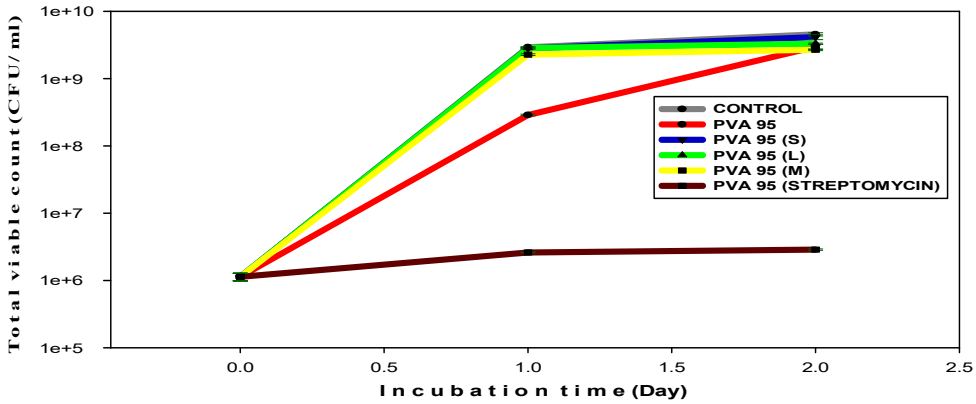

Figure 4: Total viable count of pure and blend films of PVA 95%. Each point is the mean ± SE (N =3)

Coliform Count

Coliform count (CC) is used as a measure of *E. coli* bacteria present on the films. *E. coli* is a harmful bacterium that can cause bloody diarrhoea and severe anaemia which may even lead to death. Microbial solution containing bacteria is inoculated and spread on Violet Red Bile agar. The bacteria colonies are allowed to grow on the nutrient medium until it becomes visible to the naked eye and the number of colonies on the plate can then be counted and expressed as colony forming units (CFU).

Keeping with the trend, Figure 5 and Figure 6 show that blend films of PVA 99-100 – streptomycin and PVA 95 – streptomycin have the lowest number of *E. coli* compared to other blend films. The results also show that pure PVA films have the highest number of *E. coli* which is to be expected because they don't have the means to inhibit the growth of bacteria. Blend films of *C. nutans* mixture and leaves seem to be the best performing film after 1 day of incubation but blend films of *C. nutans* mixture, leaves, and stems were not significantly different after 2 days of incubation.

Overall, none of the films perform better than blend films of streptomycin in terms of antimicrobial inhibition because streptomycin is a strong antibiotic against bacteria. Streptomycin works by disrupting the bacteria from making its own protein therefore causing its death [12].

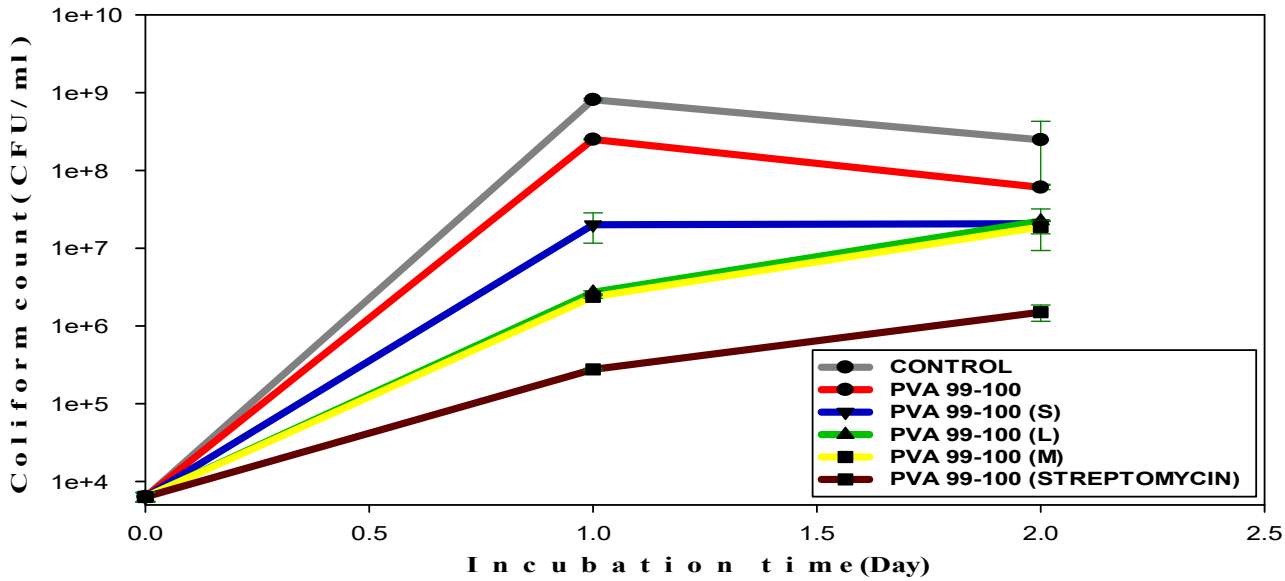

Figure 5: Coliform count of pure and blend films of PVA 99-100%. Each point is the mean ± SE (N =3)

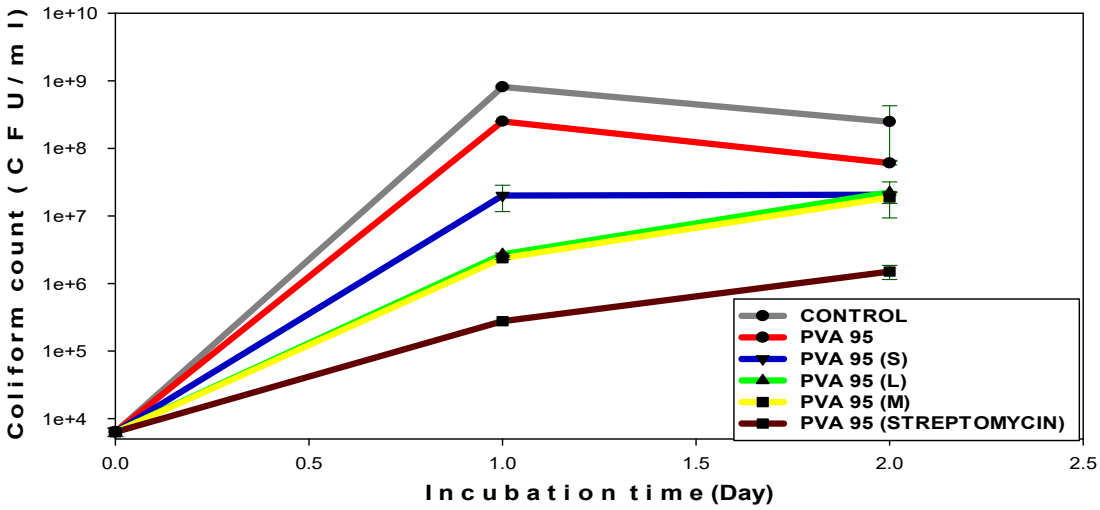

Figure 6: Coliform count of pure and blend films of PVA 95%. Each point is the mean ± SE (N =3)

Total Chlorophyll Content (TCC)

The total chlorophyll content is used as a measure of colour of the pure and blend films. Films blended with *C. nutans* flour have a greenish tinge as shown in Figure 1 which is due to the chlorophyll content in *C. nutans*. Acetone is used as the solvent as it allows for sharp absorption peaks for chlorophyll which eases its quantification. The light absorbance observed was proportional to the chlorophyll content in the sample.

Figure 7 shows the absorbance peak values for the pure and blend films. The largest value was observed for the blend film of PVA 99-100 – *C. nutans* leaves. In contrast, PVA 99-100, PVA 95, PVA 99-100 – streptomycin, and PVA 95 – streptomycin films have low absorbance values. This result was indeed expected because these films lack a dark green tinge which would have indicated the presence of chlorophyll.

The intensity of the dark green colour was dependent on the chlorophyll content of the *C. nutans* in the blend films. In this case, blend films with *C. nutans* leaves have a stronger colour compared to the *C. nutans* stems and *C. nutans* mixture blend films. This is because leaves contain a higher content of chlorophyll than stems [15]. Even though the PVA 99-100 – *C. nutans* leaves film have the highest chlorophyll content, it did not resulted in better antimicrobial performance and PVA 99-100 – *C. nutans* mixture is still regarded the best performing film.

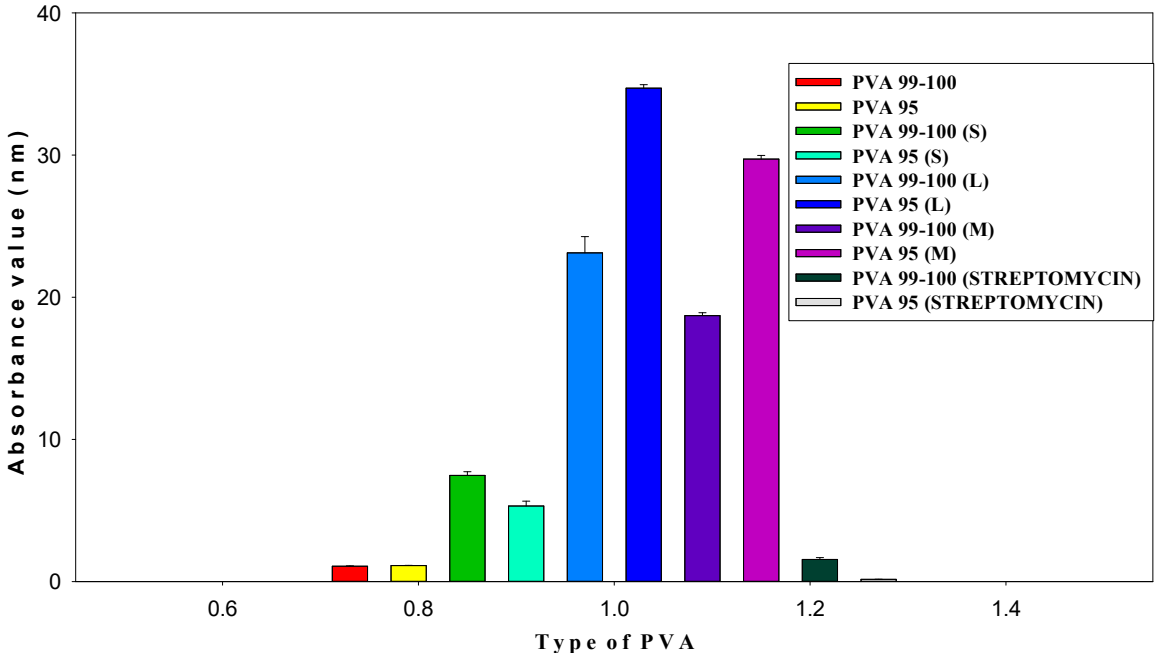

Figure 7: Absorbance value of the pure and blend films

Correlation between Total Viable Count (TVC), Coliform Count (CC) and Total Chlorophyll Content (TCC) of the Blend Films

Principle component analysis (PCA) was employed to describe correlation between TVC, CC and TCC of the blend films studied. Means of all the data were plotted against the first two components (Figure 8). The first component, accounting for 61.8% of total variation, separated PVA with *C. nutans* from blend PVA with streptomycin. The second component, accounting for 25.90% of total variation, separated pure PVA from blend PVA with streptomycin.

Correlation of the attributes generated from the data with first two dimensions (first dimension horizontally, second dimension vertically) for all the attributes tested are shown in Figure 9. The first component was associated with most of the attributes tested. Samples to the left have higher TVC and TCC. The second component was associated with CC. Samples towards the top (PVA 95) have higher CC compared to the samples towards bottom (PVA 99-100, PVA 99-100(M), PVA 99-100 – Streptomycin and PVA 95 – Streptomycin. Since all this samples (PVA 99-100, PVA 99-100 – *C. nutans* mixture, PVA 99-100 – Streptomycin and PVA 95 – Streptomycin were grouped together, it can be concluded that PVA 99-100 – *C. nutans* mixture blend film was the best film for antimicrobial activity regardless the content of TVC, TCC and CC.

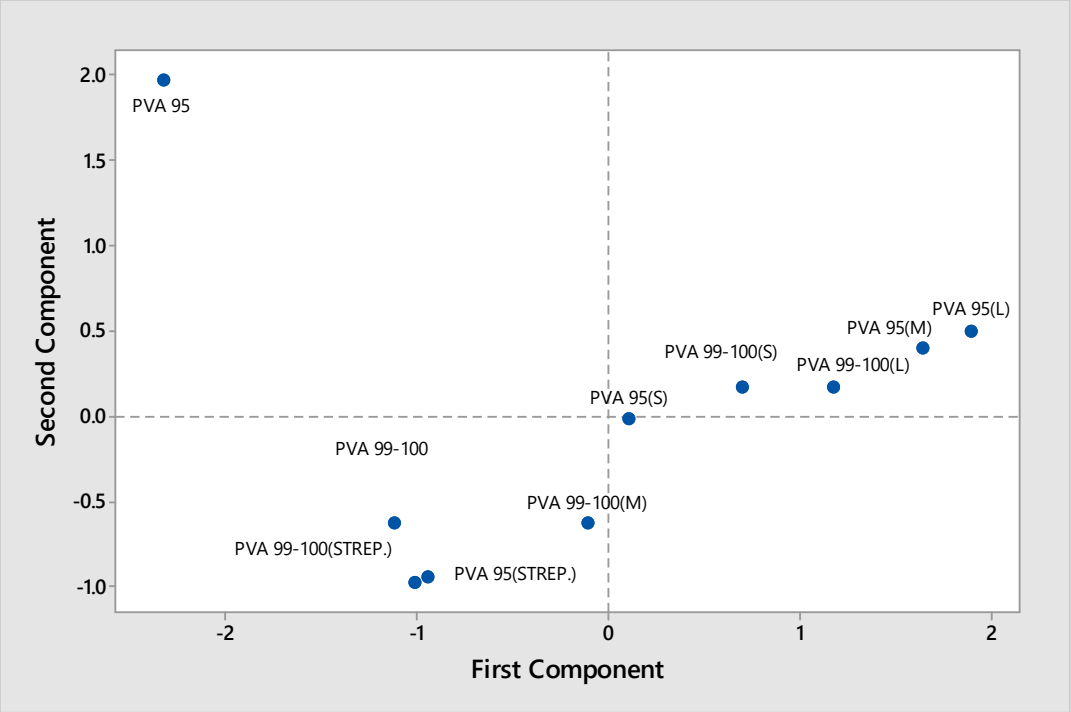

Figure 8: Score plot of PCA analysis

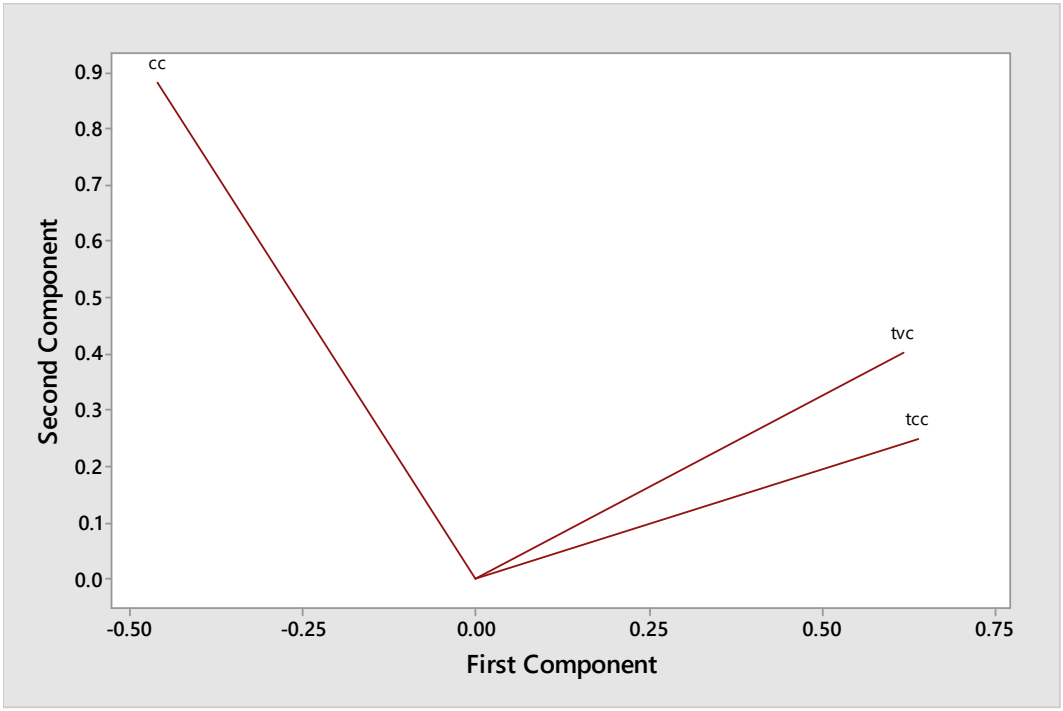

Figure 9: Loading plot of PCA analysis

Characterization of Films

Thickness

The thickness of blend films of PVA 99-100 - *C. nutans* mixture and PVA 99-100 – streptomycin was measured using a calliper and the results was tabulated in Table 2. The thickness of PVA 99-100 – *C. nutans* mixture films was measured to be from 0.15 to 0.20 mm, whereas the thickness of PVA 99-100 – streptomycin films is measured to be from 0.02 to 0.05 mm. The thicker PVA 99-100 – *C. nutans* mixture films were thought to be due to the particle size of *C. nutans* flour being larger than that of streptomycin [16].

Table 2: The thickness of blend films of PVA 99-100%

Type of blend film	Thickness (mm)
PVA 99-100 - *C. nutans* mixture	0.15±0.05
PVA 99-100 – streptomycin	0.02±0.03

Tensile Strength and Elongation at Break

The mechanical properties of the films (PVA 99-100 –streptomycin and PVA 99-100 – *C. nutans* mixture) were assessed by measuring their tensile strength (TS) (Figure 10) and elongation at break (E) (Figure 11). PVA 99-100 – *C. nutans* mixture blend films were found to have better tensile strength and elongation at break compared to streptomycin blend films. This means that PVA 99-100 – *C. nutans* mixture blend films were more flexible than streptomycin blend films. The superior tensile strength of PVA 99-100 – *C. nutans* mixture blend films was thought be due to the OH-groups of phenolic compounds in *C. nutans* forming hydrogen bonds with each other. It must be noted that SEM images show that the surface of the blend film was porous [17]. Another factor was the use of glycerol as plasticizer for the blend films of PVA 99-100 – streptomycin. Glycerol is highly hygroscopic which attracts water molecules into between the chains of adjacent macromolecules. This increases the free volume in the film which in turn reduces its mechanical strength [8].

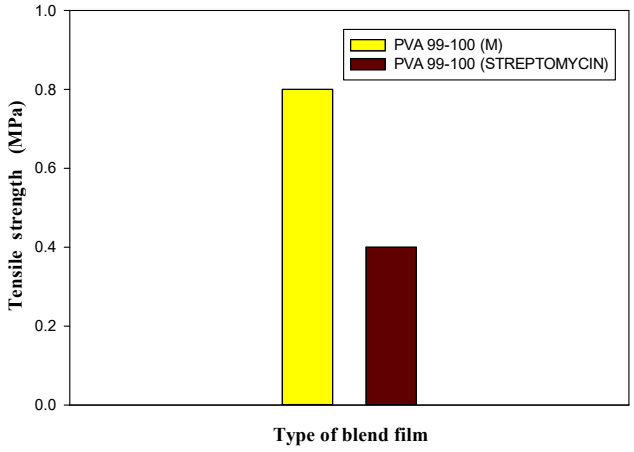

Figure 10: Tensile strength of the blend film

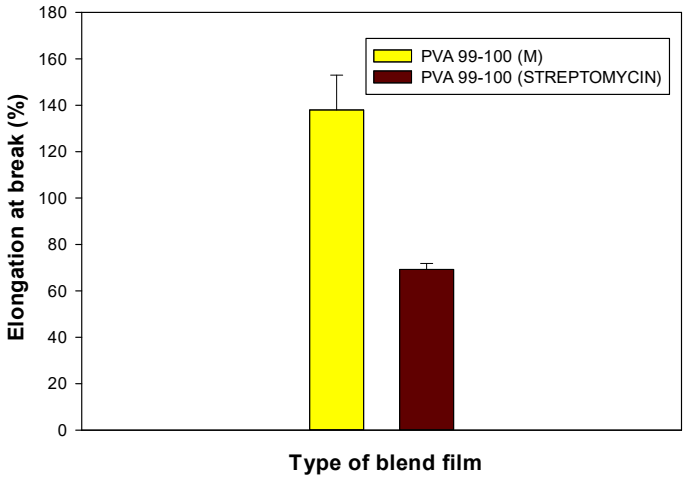

Figure 11: Elongation at break of the blend film

Surface morphology and cross-sections

Scanning electron microscopy was used to study the surface morphology and cross sections of PVA 99-100 – streptomycin and PVA 99-100 – *C. nutans* mixture. The surface morphology was affected by the porosity and particle size of the components. The cross-sections of the blend films were studied to analyse the microstructure of the films.

The SEM for the blend film of PVA 99-100 – streptomycin revealed that the film was non-porous and mostly featureless with some uneven spots (Figure 12: (E) – (H)). Under 1000x magnification, the surface of *C. nutans* mixture blend films show randomly distributed micro-cracks and branch like structure with large spaces in between (Figure 12: (A)). This is due to the improper crystallization of *C. nutans* particles in the film.

The microstructure of the film is the result of the interactions between the polymers in the blend. The cross-sections shown in Figure 13 reveal a continuous and homogeneous phase for both blend films. PVA 99-100 – *C. nutans* mixture blend films have branched-like structures which are revealed to be lignin though FTIR analysis [19]. Cross- section images of PVA 99-100 – streptomycin blend films reveal aggregated crystalline structures.

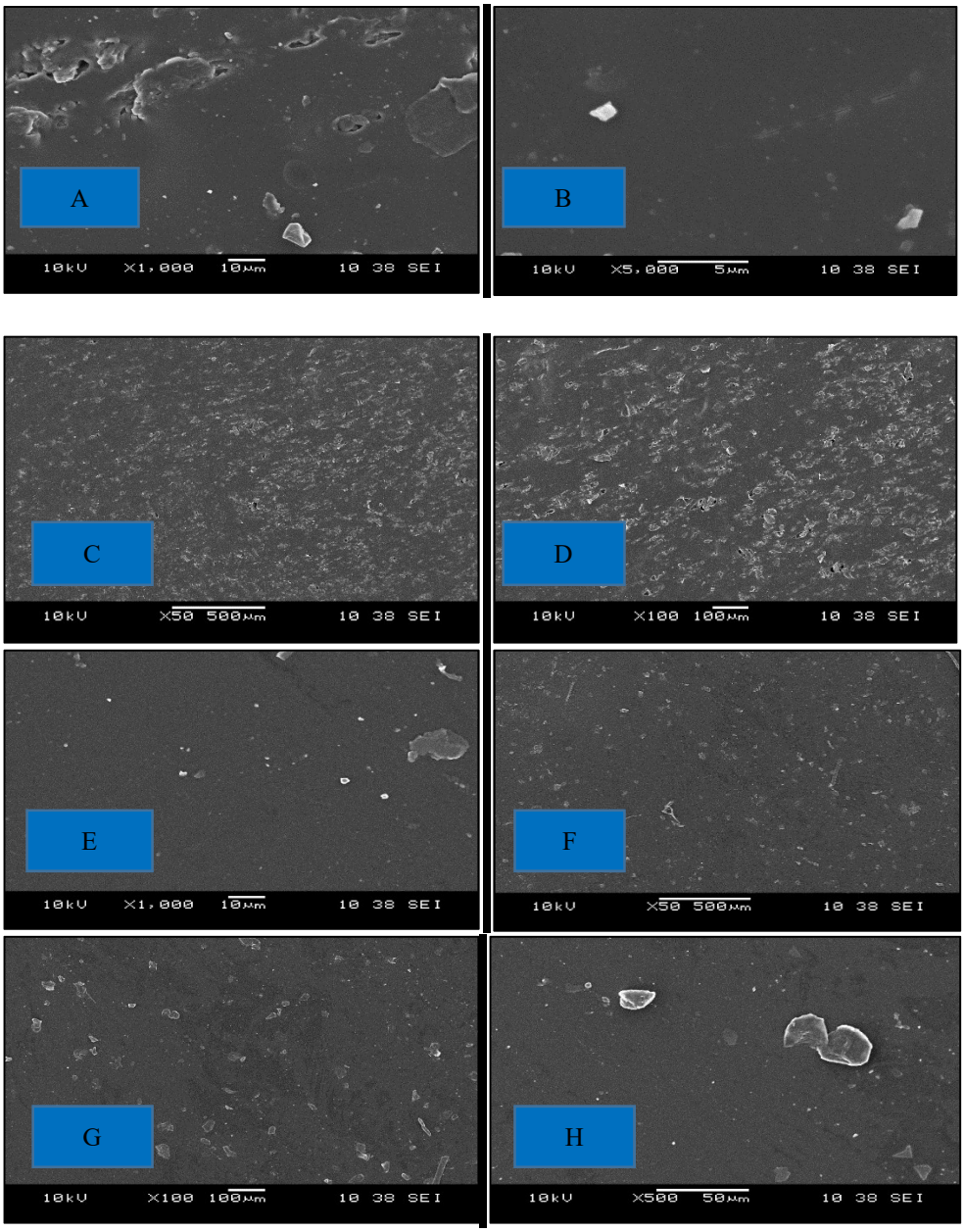

Figure 12: SEM images of surface morphology of the blend films of PVA 99-100%: (A) 1000x magnification of blend film of PVA 99-100 - C. nutans mixture (pores and cracky), (B) 5000x magnification of blend films of PVA 99-100 – C. nutans mixture, (C) 50x magnification of blend films of PVA 99-100 – C. nutans mixture (small pores and cracks visible), (D) 100x magnification of blend films of PVA 99-100 – C. nutans mixture (small pores and cracks visible), (E) 1000x magnification of blend films of PVA 99-100 – streptomycin (no pores, smooth and uneven surface), (F) 50x magnification of blend films of PVA 99-100 – streptomycin (no pores, smooth and uneven surface), (G) 100x magnification of blend films of PVA 99-100 – streptomycin (no pores, smooth and uneven surface), (H) 500x magnification of blend films of PVA 99-100 – streptomycin (no pores, smooth and uneven surface)

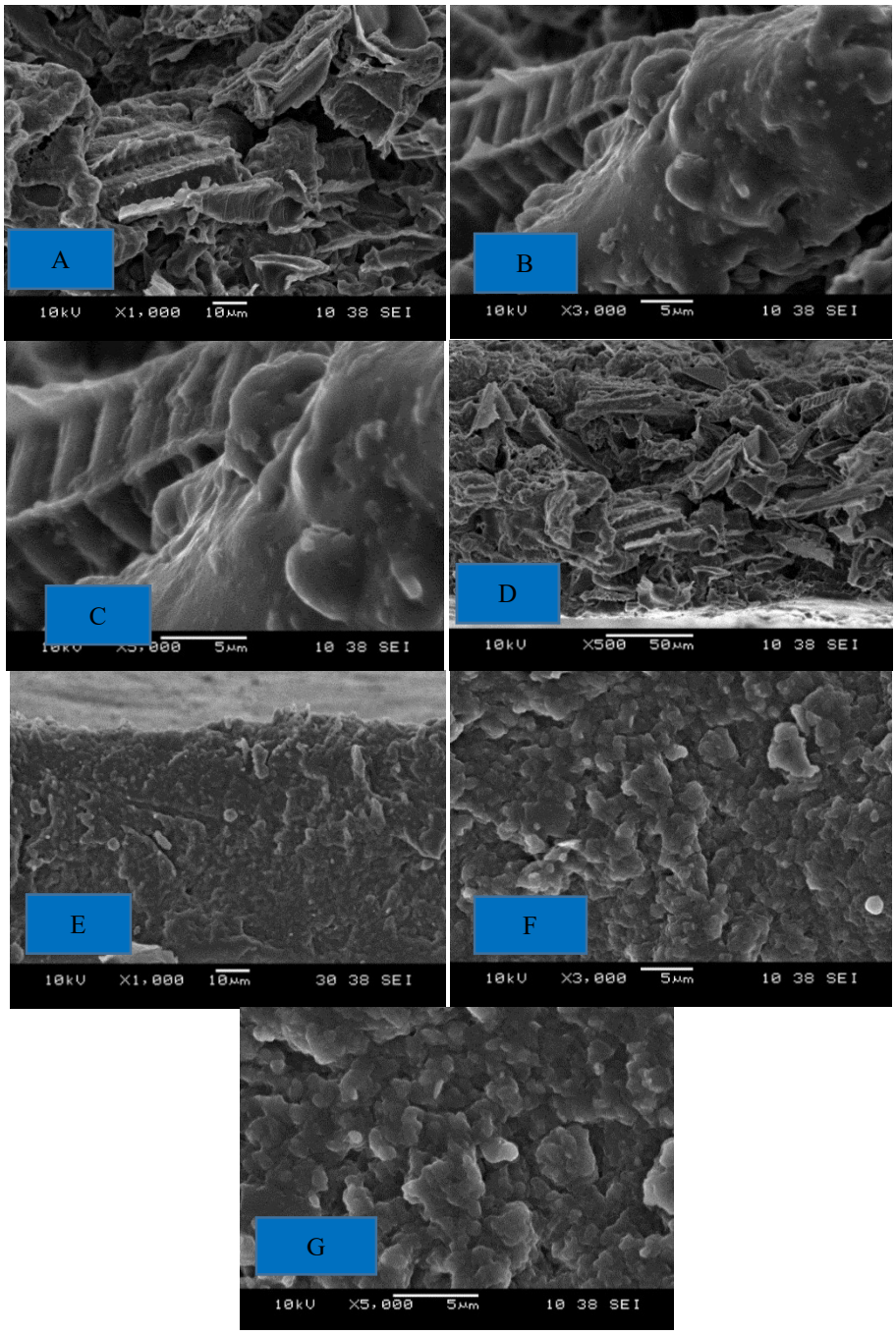

Figure 13: SEM images of cross sections of the blend films of PVA 99-100%: (A) Blend film of PVA 99-100 - *C. nutans* mixture (branch),1000x, (B) lignin structure of blend films of PVA 99-100 – *C. nutans* mixture (branch), 3000x, (C) blend films of PVA 99-100 – *C. nutans* mixture (branch), 5000x, (D) blend films of PVA 99-100 – *C. nutans* mixture (branch), 500x, (E) blend films of PVA 99-100 – streptomycin (irregular), 1000x, (F) blend films of PVA 99-100 – streptomycin (irregular), 3000x, (G) blend films of PVA 99-100 – streptomycin (irregular), 5000x

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis is used to detect the presence of functional groups in the blend films. Table 3 assigns functional groups to major peaks observed in the spectra for pure PVA and blend films. Absorption bands that were found in the range of 4000 – 2000 cm^{-1} correspond to vibrational frequencies of specific functional groups and the region from 2000 to 650 cm^{-1} is the fingerprint region, being highly specific to each taxon.

Table 3: FTIR peak assignment of PVA and *C.nutans* flour-PVA blend films

Component	Wavelength (cm^{-1})	Assignment
PVA 99-100% – Glycerol	3339.33	O-H stretching
	2943.65	C-H stretching
	1652.88	C=C stretching
	1421.69	O-H and C-H bending
	1217.43	CH_2 bending
	1109.79	C-O stretching
	1042.38	C-OC stretching
	924.49	C-O symmetric stretching
	853.80	CH_2 rocking
PVA 99-100% - Mixture	3326.14	O-H stretching vibration of PVA with a secondary amine (-NH)
	2945.22	CH_3, CH_2 and CH
	1647.05	Alkenes
	1419.89	Aromatics
	1042.20	Aliphatic amines, alcohols, carboxylic acid, esters and ethers
	925.90	Aromatics
	849.13	Alkyl halides, alkenes, primary secondary amines, and aromatic ring
PVA 99-100% - Streptomycin	3324.60	O-H stretching
	2944.02	C-H asymmetric
	1652.01	C=C stretching
	1417.93	O-H and C-H bending
	1110.65	C-O stretching
	1041.93	C-OC stretching
	922.43	C-O symmetric bending

Figure 14 shows the FTIR spectrum of blend films of PVA 99-100 – streptomycin, blend film of PVA 99-100 – C. nutans mixture and blend film of PVA 99-100. For pure PVA, the absorption band at 3339.33 cm^{-1} corresponds to O-H stretching due to strong intra- and inter-molecule hydrogen bonding. The strong band at 2943.65 cm^{-1} is attributed to C-H stretching mode [20]. The band at 1652.88 cm^{-1} is attributed to C=C stretching mode and the band at 1421.69 cm^{-1} is attributed to O-H and C-H bending. The band at 1217.43 cm^{-1} is due to H-C-H bending while the

band at 1109.79 cm^{-1} is due to C-O stretching and the band at 1042.38 cm^{-1} is due to C-O-C stretching. Also, the band at 924.49 cm^{-1} is assigned to the C-O symmetric stretching mode and the band at 853.80 cm^{-1} is assigned to the C=C stretching mode [21].

For blend film of PVA 99-100 - *C. nutans* mixture, the band at 3326.94 cm^{-1} is assigned to N-H stretching in amine, and the band at 2945.22 cm^{-1} is due to C-H asymmetric or symmetric stretching vibrations of alkanes (CH_3, CH_2 and CH). The strong band at 1647.05 cm^{-1} is attributed to alkenes or the stretching of C=C. The band at 1419.89 cm^{-1} is attributed to C-C stretching vibrations in an aromatic ring. The band at 1042.20 cm^{-1} is then attributed to carbohydrate moieties, especially starch while the band at 849.13 cm^{-1} is attributed to the presence of alkyl halides, alkenes, primary amines, secondary amines, and aromatic compounds [19].

The band at 3324.60 cm^{-1} is attributed to O-H stretching, 2944.02 cm^{-1}, to asymmetric C-H stretching, 1652.01, C=C stretching, and 1417.93 cm^{-1} to the bending of O-H and C-H. The band at 1110.65 cm^{-1} is attributed to C-O stretching while the band at 1041.93 cm^{-1} is attributed to C-O-C stretching. The band at 922.43 cm^{-1} is due to the symmetric bending of C-O and the band at 854.16 cm^{-1} is due to the rocking of CH_2 was located at [21].

Both starch and PVA possessed abundant –OH and C–O groups, which could form hydrogen bonding and improve the compatibility between then and increase the strength of the films [22]. Present of alkyl halides, alkenes, primary secondary amines, and aromatic ring in the PVA 99-100 - *C. nutans* mixture might be contribute to the antimicrobial properties of the film [23].

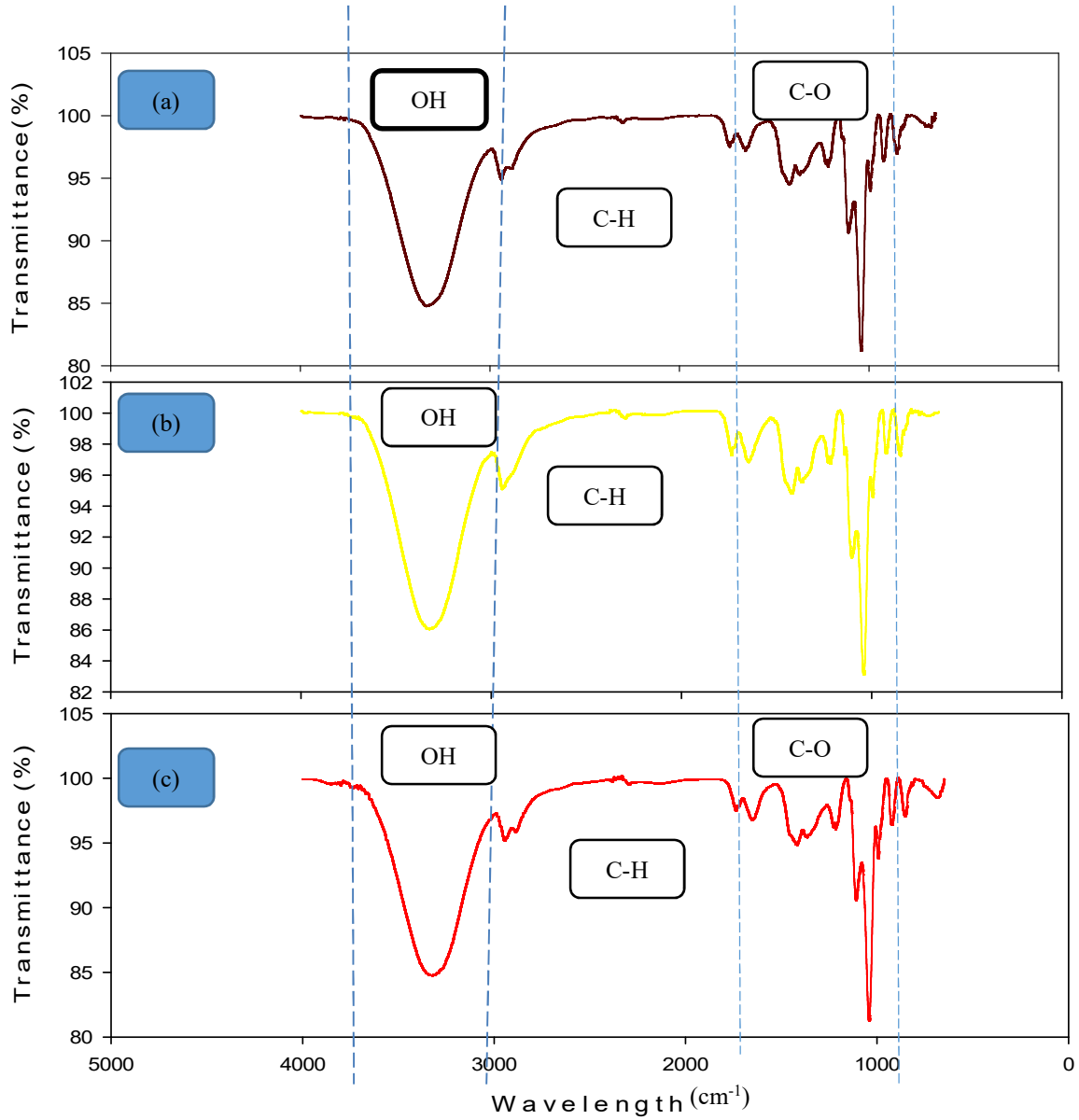

Figure 14: FTIR spectrum of blend films: (a) blend film of PVA 99-100 – streptomycin, (b) blend film of PVA 99-100 – *C. nutans* mixture, (c) blend film of PVA 99-100

Conclusion

Antimicrobial films were successfully developed using PVA 99-100%, PVA 95%, and PVA 88% but not PVA 75%. Pure and blend films made using PVA 75% cannot be peeled smoothly and easily crack making them not suitable for film-forming. The meat model shows that all pure and blend films of PVA 99-100% and PVA 95% were suitable for use as food packaging except for PVA 88% films which becomes sticky when in contact with the meat due to its higher solubility and low water resistance. Based on the PCA analysis, PVA 99-100 – *C. nutans* mixture was suggested as the best antimicrobial film and comparable with the control film (PVA 99-100 – streptomycin). Surface morphology of the films have a great influence towards its properties. *C. nutans* mixture blend film consist of branch-like lignin structures while PVA 99-100 – streptomycin blend film has a rough surface with dispersed crystalline structures. This might be the reasons why the strength of PVA 99-100 – *C. nutans* mixture are better than PVA 99-100 – streptomycin film. This study shows that, blend film of *C. nutans* flour and PVA have a huge potential to be explored and commercialized as food packaging in the near future. However, the formulation of antimicrobial films should be improved by manipulating flour proportions of *C. nutans* to make it comparable to the current film available in the market.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

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