

Evaluation of the physicochemical and antimicrobial properties of the polyvinyl alcohol-mulberry leaf extract blend film

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1 **Evaluation of the physicochemical and antimicrobial properties of the polyvinyl alcohol-**
2 **mulberry leaf extract blend film**

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1 **ABSTRACT**

2 In this study, mulberry (*Morus nigra sp.*) leaves extract (MN) was used as a structure modifier and
3 antimicrobial agent in poly(vinyl alcohol) (PVA) blend films. We prepared PVA/MN blend films
4 by solution casting at various concentrations. The SEM micrographs showed that PVA_MN75 has
5 a smooth and continuous cross-sectional morphology with better compatibility. Fourier-transform
6 infrared spectroscopy results suggest that a specific concentration of MN in PVA films
7 facilitates a physical interaction that improves mechanical and thermal properties. The tensile
8 strength of the films increased from 1.89 MPa to 2.91 MPa after adding mulberry leaf extract.
9 Furthermore, the films were assessed for their solubility in water (12.12-15.83 %), absorption
10 (9.80-21.61 %) and moisture content (6.53-14.26), which further demonstrates that blend films
11 exhibit lower water solubility. Moreover, the soil burial biodegradation test indicates that
12 PVA/MN film biodegradability is strongly affected by its MN concentration. Upon soil burial, all
13 films showed an increase in biodegradability of 29% as burial time has prolonged in both soil and
14 compost for 37 days. Enhanced antibacterial activity has observed in the PVA/MN film-forming
15 solution and can be considered as a promising substitute for polluting plastic packaging.

16 **Keywords:** Biopolymers, Biodegradable Films, Polyvinyl Alcohol, Biodegradation, Antibacterial.

17

1 INTRODUCTION

2 Nowadays, a technological breakthrough in the packaging is reflected on the development of food
3 packaging, pharmaceutical, biomedical and other packaging industries. The industrial revolution
4 has brought packaging materials to develop within the areas of edible, biodegradable,
5 nanocomposite, active and intelligent packaging.[1-5] Flexible film packaging types describe any
6 type of materials which is not rigid, but the term “flexible polymer” is often reserved for non-
7 fibrous plastic polymers which are composed of molecular chains have long sequences of one or
8 more species of atoms or groups of atoms linked to each other.[6,7] Poly(vinyl alcohol) (PVA) is
9 a synthetic polymer carries the predictable properties of hydrophilic, biodegradable, tasteless,
10 odorless, good mechanical properties and high ability to form packaging films.[8,9] Due to the
11 predictable hydrophilic properties, PVA need to bind with other chemicals to promote a change in
12 physical properties and to alter their hydrophilicity nature to hydrophobic properties, by removing
13 hydroxyl groups between the polymer chains. Theoretically, all chemical compounds that are able
14 to react with hydroxyl groups could be used as potential structure modifying (crosslinker) agent.
15 PVA react with common crosslinking agents like dicarboxylic acid, tricarboxylic acid, dialdehyde,
16 aldehyde, phenol group, carboxylic acid in ethylene glycol di-methacrylate and lactic acid to alter
17 the hydrophilic properties.[10-16,5]

18 A molecule's size is crucial to the formation of a PVA-composite film because smaller molecules
19 can readily fit into the void spaces within the polymer. Indeed, glyoxal and glutaraldehyde (GA)
20 have an excellent potential for forming crosslinked composite films and exhibit antimicrobial
21 properties, and are widely used in disinfectants.[17,13,16,14,15] Poly ethylene glycol
22 diacrylchloride adapted the structure of PVA to offer tremendous biotechnology and biomedical
23 potential, whereas butyl glycidyl ether (BGE) modified PVA for use as a surface sizing agent in

1 paper manufacturing. [18,19] The microbial activity also can be inhibited through other natural
2 plant and herbs extraction, as reported neem, guava, indian acalypha, green chiretta and Holy basil
3 leaves extraction enhanced the inhibition of microbial growth [20-22,7,23-29]. In addition, natural
4 sources such as cinnamaldehyde and syzygium cumini leaves extract have been successfully
5 applied to change the structure of PVA and at the same time minimize the inhibition of microbial
6 growth of *Escherichia coli* species. [24,25] Moreover, turmeric and clove oil are widely known as
7 effective antimicrobial agents against foodborne pathogen.[23,27]

8 Mulberry, or known as *Morus nigra* sp. leaves are widely used in Chinese traditional medicine to
9 fight against certain disease and it has not yet been studied in food and packaging technology. *M.*
10 *nigra* sp. is one of the mulberry species that can be found in western Asia. The mulberry leaves
11 extract (MN) could be a potential agent to alter the structure of PVA and also an antimicrobial
12 agent. The main constituents of *M. nigra* sp. are avones, steroids, essential oil and other
13 constituents. It has been proved that MN possess anti-hypertensive, anti-hyperlipidemic, anti-
14 hyperglycemic, anti-aging and antioxidant activities.[22,30] Beside of having pharmacological
15 effects, the acidic compound in MN can act as a profiling and antioxidant potential.[20] Several
16 studies also demonstrated that the chemical compounds obtained from mulberry leaves after the
17 extraction process can contribute to antimicrobial activities based on the plant's species, harvest
18 period and processing.[31,32] In light of the high potential of MN in food packaging technology
19 especially in PVA filming, the present work has conducted to evaluate the influence of MN on the
20 morphological, mechanical, thermal and water solubility properties of PVA films prepared by
21 physical blending. Additionally, the biodegradability and antimicrobial test were performed to
22 further access the potential of the films produced.

23

1 **EXPERIMENTAL**

2 **Materials**

3 PVA (99.8%-100% hydrolyzed) was purchased from Sigma Aldrich. Mulberry leaves were picked
4 from garden in Putrajaya, Malaysia. Glycerol was supplied by Emsure. Methanol and hydrochloric
5 acid (HCl) were provided by Merck. All the aqueous solutions throughout this work were prepared
6 using deionized water.

7 8 **Methods**

9 **Extraction of Mulberry Leaves**

10 In a typical synthesis process, fresh mulberry leaves with a width of 10 to 15 cm were picked from
11 their plant. The leaves were washed to remove any dust. Then, the mulberry leaves were dried in
12 oven at 80 °C. After 5 h, the leaves were taken out from the oven and left cooled for 10 min. The
13 dried leaves were grinded using a grinder until fine powder was obtained. Then, 20 g of leaves
14 powder is poured into a conical flask containing 80:20 v/v of methanol and distilled water. The
15 solution was put in the orbital shaker for 8 h at room temperature. After that, the solution was
16 filtered using Whatman No.1 filter paper. The solid residue obtained after filtration were extracted
17 twice with fresh solvent. The MN obtained were isolated from the solvent using a rotary evaporator
18 at 45 °C under a reduced pressure. The MN was stored at 4 °C for further use.

19 20 **Preparation of PVA/MN Blend Films**

21 PVA powder was dissolved in 45 mL deionized water at 160 °C for 2 h. The PVA solution was
22 left cooled for 5 min. Then, 1% of glycerol plasticizers was added into the solution by using a
23 disperser (IKA, T-18 Digital Ultra-Turrax) for 2 min. After that, 0.65, 0.75 and 1 mL of MN was

1 added into the solution and was mixed for 3 min, and followed by the addition of the structure
2 modifying agent obtained from evaporation process. Finally, HCl was added into the mixture for
3 3 min to increase the transparency of the solution. The solution was left to allow the escape of
4 bubbles before being casted. The film was then casted using Doctor Blade and left dried overnight.
5 The dried films were peeled off and placed into a desiccator. The thickness of the films was
6 measured with a digital micrometer (Mitutoyo IP 65, Japan) for at least five positions of each
7 random sample. All the PVA/MN blend films prepared were denoted as PVA_MN_x where, X is
8 the content of MN added (0.65, 0.75 and 1 mL).

9 **Morphology and Mechanical Characterization**

10 The morphologies of the cross sections of the PVA/MN blend film produced were studied using a
11 scanning electron microscopy (SEM) (Hitachi, S-3400N, Japan). A universal tensile tester
12 (Instron, 5582Q4970) was used to measure the mechanical properties of the films, as per ASTM
13 D882 standard discussed elsewhere. In the tensile strength study, 25.4 mm × 100 mm strips with
14 a gauge length set at 50 mm was used and tested in triplicates. The spectral analysis was conducted
15 using a Fourier-transform infrared spectroscopy (FTIR) (Thermo Scientific, Nicolet IS10) at range
16 from 4000 cm⁻¹ to 400 cm⁻¹. The thermogravimetric analysis (TGA) curves for the polymer
17 samples were recorded using a TGA (Perkin Elmer, STA8000) by heating the films at rate of 30
18 °C/min under nitrogen atmosphere with temperature range from 20 °C to 800 °C.

19 **Moisture Content, Water Solubility and Water Absorption Properties**

20 The moisture content (MC) of PVA/MN blend films was determined by measuring the weight loss
21 of films, after drying in an oven at 105 °C until a constant weight was obtained. Samples were
22 tested in triplicates and results were expressed as an average of the percentage of MC of samples
23 that was calculated using Eq. (1).

$$MC (\%) = \frac{W_{0,dry} - W_{1,dry}}{W_{0,dry}} \times 100 \% \quad (1)$$

1 where $W_{0,dry}$ is the initial weight of the sample and $W_{1,dry}$ is the final weight of sample after drying
2 process.

3

4 Besides, the water solubility (WS) of the prepared films in water was studied in the term of the
5 loss of the dry matter of film after solubilized and immersed in water for 24 h at room temperature.
6 After 24 h, the moisture on the surface of the films was removed and dried in oven until they
7 maintained at a constant weight. The WS was calculated using the Eq. (2).

$$WS (\%) = \frac{W_{0,sol} - W_{1,sol}}{W_{0,sol}} \times 100 \% \quad (2)$$

8 where, $W_{0,sol}$ is the initial weight and $W_{1,sol}$ is the final weight of the film after immersion and
9 drying steps.

10

11 The water absorption (WA) of PVA/MN films was determined by immersing the films prepared
12 in an acidic 0.2M of H_2SO_4 solution at room temperature for 24 h. The procedures were modified
13 from method reported. After that, the moisture on the surface of the films was removed and the
14 weight of the film was measured. The WA was calculated using the Eq. (3).

$$WA (\%) = \frac{W_{1,abs} - W_{0,abs}}{W_{1,abs}} \times 100 \% \quad (3)$$

15 where, $W_{0,abs}$ is the weight before absorption and $W_{1,abs}$ is the weight of the swelled film.

16

17 **Biodegradability Test**

1 The soil burial test was used to evaluate the biodegradability of the films. The films were buried
2 in composted soil at a depth of 10 cm with size of 20 × 20 mm. The soil with samples were left in
3 laboratory and maintain at sparkling water with regular interval time. The degradation rate of the
4 films was determined in terms of the weight loss of the sample over time.

6 **Antimicrobial Test**

7 To further access the capability of the prepared PVA/MN blend films in resisting the bacteria
8 growth, their antimicrobial activity against *Escherichia coli* and *Legionella* bacteria were studied
9 by using agar disk diffusion test, or alternatively known as Kirby-Bauer antibiotic test. The agar
10 plate was first inoculated with microbial strains. A 5 mm hole was made on agar surface by using
11 a cork borer and the PVA/MN film making solution was transferred carefully into the small hole.
12 After that, the petri dish was incubated in an incubator at 37 °C for overnight, and the microbial
13 growth on the agar plate was observed.

15 **RESULTS AND DISCUSSION**

16 **Morphology, Mechanical and Thermal Characteristics of the PVA/MN Blend Films**

17 Figure 1 shows the SEM images of the cross section of the selected films. The SEM image of
18 PVA/MN film showed compact and smooth structure. However, as increase the concentration of
19 MN extraction, the image of cross sectional is varied. PVA_MN₆₅ shows compact and rough cross
20 section. Meanwhile, PVA_MN₁₀₀ shows irregular pores or cavities distributed through the PVA
21 matrix. These can be attributed to the lipid or creamy aggregation during drying steps. This also
22 can prove that lipid of glycerol dispersed in the polymer matrix led to a discontinuous and loose
23 structure in the matrix, compare with that PVA_MN₇₅ that shows compact smooth and continuous

1 cross section. In facts, PVA in the composition itself makes the structure of films softer and more
2 flexible. Obviously, a non-uniform morphology of the microstructure which can be caused by the
3 phase separation and this non-uniform irregular pores distributed tends to reflect a decrease in
4 intermolecular interaction between MN and PVA matrix that lead to the decrease in mechanical
5 properties of the blend films.[26] In this study, the most important effect of MN when it presents
6 at certain blend formulation, indicating the heterogeneous phase morphology. This phenomena has
7 occurred owing to that it might be due to the aggregation of the extraction and plasticizer lead to
8 the loss of integrity which disrupt the formation of intermolecular hydrogen bonding resulted in
9 weak intermolecular interaction lead to increase the flexibility in the films.[25]

10 It was observed that the transparency of PVA/MN blend films slightly decrease when the amount
11 of MN increased, particularly in the films consisting of 1.0 mL MN (PVA_MN₁₀₀). Conversely,
12 this PVA/MN blend films remained transparent at high MN concentration.[24] Meanwhile, the
13 film forming solution produced a homogeneous solution with transparent, odorless and easy to
14 handle after drying steps. The thickness of the films was not sensitive to the changes of MN
15 concentration, as presented in Table I. This due to release of alcohol and phase separation during
16 the drying steps. However, the thickness of PVA/MN films increase when the content of MN
17 reached to 0.75 (PVA_MN₇₅). Overall, the thickness of the films varied between 0.195mm and
18 0.082mm. A small value of standard deviation on all of the measurement demonstrated the films
19 uniformity.

20 The mechanical properties of the cross-linked PVA film at different concentration are presented
21 in Table I. From Figure 2, the tensile strength (TS) of the films decreases with increasing the
22 concentration of MN extraction after it reach optimum content. The optimum content of MN
23 extraction in the PVA/MN blend was optimized at 0.75 (PVA_MN₇₅) where it gives the maximum

1 properties of TS from 1.886 to 2.907 MPa and tensile modulus (TM) from 0.175 to 0.113 MPa.
2 After 0.75 concentration of PVA_MN film, the tensile strength reduced. The decreasing TS (2.907
3 MPa to 1.608 Mpa) and increasing TM (0.113 MPa to 0.205 MPa) can be relate from the cross-
4 sectional image of SEM where the film exhibits irregular pores distribution that lead to increase
5 brittleness in the films. The increased TS from 1.886 MPa to 2.907 MPa is attributed to an increase
6 in MN content. Which lead to an increase in the cross-linkage between the MN extraction and
7 PVA matrix through intermolecular –OH and hydrogen bonding. A similar study had suggested
8 as increase in tensile strength (TS) with decreasing tensile modulus (TM) is due to the
9 enhancement of structural bonds in the polymer network which it reduce the brittleness properties
10 of PVA polymer.[33,34] This study found that the cross-linking and concentration of MN
11 extraction affected TS and TM value of cross-linked films. The cross-linking reaction allowed the
12 MN extraction to combine with PVA which improves mechanical properties.

13 Additionally, TGA was conducted to study the thermal stability of the different concentration of
14 the MN in PVA plastic films. As illustrated in Figure 3(A), the PVA/MN blend films incorporated
15 with different concentration of MN showed as increasing the concentration of extraction, the
16 region of mass loss will shift towards higher temperature range. The initial mass loss of
17 PVA_MN₆₅ films in the region from 50 to 80 °C resulted from the evaporation of water.
18 Meanwhile, as increase the concentration of extraction the evaporation of water take place at
19 region from 50 to 140 °C. It is know that the second thermal degradation is between 250 and 420
20 °C is primarily due to the pyrolysis of PVA. On the other hand, when the concentration of MN
21 increased from 0.75 until 1.0, the onset decomposition temperature was about 300 °C. This is
22 shown that the thermal decomposition of the films shifted toward a higher temperature range
23 compared to low extraction concentration PVA film. Improved thermal stability in the PVA/MN

1 blend film was observed where the third stage mass loss occurred above the region of 400 °C.[24].
2 This enhancement in the thermal stability and change in the decomposition patterns of the films
3 may resulted from the structural alternation caused by the interaction of the hydroxyl group of the
4 PVA macromolecules and the surface phenolic group of MN.

5 A comparison between sample compositions was shown in Derivative thermo-gravimetric (DTG)
6 peak of Figure 3(B). The resulted data shows that the addition of MN extraction increase and
7 improves its degradation temperature. Through DTG peaks clearly can be seen that, decomposition
8 temperature of PVA films improves considerably in presence of glycerol and MN extraction as the
9 amount of the extraction increase until it reaches certain sample compositions. This is due to new
10 hydrogen bonding between the molecule of PVA, glycerol and incorporation of the natural
11 extraction. The increased DTG peak intensity and decrease peak broadness indicate that there was
12 a smaller number of –OH group present in the system of PVA matrix film incorporated with
13 glycerol and MN extraction. The lowest and narrow peak intensity were obtained from sample of
14 PVA_MN₇₅ shows lower degradation rate and better thermal stability compared other sample and
15 it were further enhanced by crosslinking of MN extraction. Which clearly indicates that the
16 hydroxyl group were engaged in the formation of hydrogen bonds between the polymer chains and
17 incorporated filler to make a strong interface between the polymer chains. The remaining of
18 hydroxyl group were participated in the crosslink reaction where the restricted chain mobility in
19 the polymer chain keeps the stiff and rigid.[35]

20 Aiming to gain better understanding for the change of properties of PVA/MN blend films, the
21 functional groups present in the PVA/MN films were identified through FTIR spectrum, as shown
22 in Figure 4. The important peaks were given in Table S1 in Supporting Information. The polymeric
23 –OH stretching appeared between 3262 cm⁻¹ and 3263 cm⁻¹, where this exhibited broad band gaps

1 indicates the inter- hydrogen bonded O-H stretching vibration of the hydroxyl group.[36,37] In the
2 PVA/MN blend film, -OH stretching vibration of PVA slightly increased to higher value 3262 cm^{-1}
3 1 and 3263 cm^{-1} , meaning that there is strong intermolecular interactions between the PVA matrix
4 and MN. This broad absorption also might due to to a rise from the O-H stretching frequency of
5 PVA and water -OH group.[38] Basically, the free hydroxyl functional group has a broad peak
6 between 3600 cm^{-1} and 3650 cm^{-1} and this broad peak will shift to peak between 3200 cm^{-1} and
7 3500 cm^{-1} when there is -OH group is engaged between the matrix and reinforcement or other
8 chemical in the formation of hydrogen bond.[39] The alkyl C-H stretching in CH_3 , CH_2 and CH
9 group and bending band from backbone chains of the matrix and chemical compound occurred at
10 2928 cm^{-1} and 1423 cm^{-1} . [36,25,35,40,37] It is also stated that between 2928 cm^{-1} and 2929 cm^{-1}
11 band gap there is a presence of methyl C-H asymmetric stretching. The presence of this group was
12 confirmed by R. Jayasekara et al. where at peak 1324 cm^{-1} attributed to the C-H stretching, bending
13 and C-O stretching of PVA.[38] The small hump at 1650 cm^{-1} is attributed to C=C stretching of
14 that aromatic compound contained in the PVA/MN blend films. Bound of water occurred at peak
15 1651 cm^{-1} due to the presence of water in the films.[38,35] Moreover, according to Murat Simsek
16 et al., a presence of band gap between 1715 cm^{-1} and 1595 cm^{-1} can be due to the symmetric -C=O
17 stretching from -COOH and -COOR (saturated ester) group.[37] Next, the absorption band
18 occurred at 1040 cm^{-1} is assigned to the C-O stretching of PVA/MN structure.[24,17,41] The most
19 appreciable changes were observed in the FTIR spectrum of PVA/MN blend films where the
20 formation of absorption peak between 1410 cm^{-1} and 1290 cm^{-1} is the primary or secondary -OH
21 stretch, phenol and tertiary alcohol OH bend. Furthermore, the cross-linking function was also
22 confirmed by the reduction of the relative intensity of hydroxyl bond in the cross-linked film.
23 Hence, the results indicated that there exists strong interaction between PVA/MN due to the

1 hydrogen bond and -OH group present in the PVA and MN extraction which supported by an
2 improvement in the mechanical properties.

3

4 **Moisture Content, Water Solubility and Water Absorption Properties of the PVA/MN Blend** 5 **Films**

6 As shown in Figure 5(A) and summarized in Table II, the water content in PVA/MN film
7 increasing as increase the concentration of MN until it reaches concentration of 0.65, then it starts
8 to decrease. This finding is attributed to the hydrophilic nature of the MN extraction and PVA
9 polymer itself due to interaction with hydroxyl group and removing of water molecule resulted
10 from the crosslink mechanisms. Moreover, glycerol and water molecule may act as a natural
11 plasticizer to allow the PVA to be more flexible than its characteristics as rigid and hard polymer
12 when in a complete dry form.[9] WS reflected the water resistance, and it may give an idea with
13 respect to films applications in packaging for food products. A very low water solubility is required
14 for food products, especially in contact with food product with high water activity that require
15 packaging integrity under ambient conditions.[41,28] The results was illustrated in Figure 5(B),
16 which cross-linked films with varied concentration of MN effected the resistance of films through
17 water. PVA_MN₇₅ shows less solubility of film towards water this may due to the cross-linked of
18 the extraction compound with PVA matrix which reduced the -OH group for water to combine
19 with the compound. It can be concluded that this natural extraction had an effective water
20 resistance, which most likely due to the establishment of hydrophobic bind and improvement in
21 rigid molecular for nation in the film thus increasing the resistivity of water.

22 WA is a measure of the amount of water absorption when material is exposed to water. As shown
23 in Figure 5(C), the PVA/MN film immersed in acidic solution gave the lower WA values where it

1 keeps decreasing as the natural extraction content increase. The phenomenon might be attributed
2 to the presence of large molecules in acidic solution making the diffusion process into film matrix
3 to be slow and producing in lower values of absorption kinetic parameters.[42] Water absorption
4 drop slightly after it reach 0.65 MN extraction content which by means it reach suitable
5 concentration of incorporation with extraction because of the hydroxyl group of PVA may be
6 cross-linked fully with the extraction and other compound in the films which it produced compact
7 crosslink structure which unable for the acidic molecule to diffuse in the matrix of films.
8 On the other hand, at concentration of 0.65, it shows higher moisture absorption which it may due
9 to some of the glycerol in films that act as plasticizer contain excess hydrophilic group in which
10 these water molecules interact with the macromolecule of glycerol by means of hydrogen bonds
11 that resulting in high moisture absorption. This phenomenon can be stated that water molecule and
12 excess hydroxyl group of glycerol can constitute a problem due their stronger plasticizer effect in
13 the films matrix and modifying the physical properties of the film.[21]

14

15 **3.4 Biodegradability and Antimicrobial Behaviors of the PVA/MN Blend Films**

16 Degradability of polymer is a crucial functionality for packaging applications. The soil burial test
17 is an assessment that gives a realistic environment, where the soil temperature, humidity, type and
18 the amount of microorganisms are in less control and differ with seasons.[21,28] The physical
19 changes of films during soil burial test as in Figure 6. The weight loss of PVA/MN films occurred
20 in soil and compost respectively. The biodegradability increases up to 29% as the burial time
21 increased in both soil and compost for 37 days. All of the films in soil and compost degraded
22 constantly in first 13 day and degraded rapidly for the next 8 days. This continuous and rapid

1 degradation was due to the composting process that occurred in two main stage as stated by
2 previous research where this stages are active composting stage and a curing period.[9,29,43-45]
3 In the 1st stage, the temperature rose and remained elevated as there is still available oxygen which
4 resulted in strong microbiology activity. In the 2nd stage, the temperature decreasing but the film
5 continued to compost but at a slower rate. In the both soil and compost, the PVA_MN₁₀₀ sample
6 showed the highest weight loss while PVA_MN₆₅ shows the lowest weight loss. This finding might
7 be attributed to the excess hydrophilic in glycerol and MN which lead to highest degradation rate.
8 Moreover, PVA itself have biodegradability properties owing to its hydrolysable, exhibited a low
9 resistance against soil burial degradation. The addition of compost help in increasing the
10 degradation rate. Figure 6 shows the weight was slightly lower for duration of 22nd to 37th days
11 However, the composting is still continuing and did not stop at a particular day. Instead of, it
12 continued slowly until the all the remaining nutrient was consumed by the survived
13 microorganisms and eventually nearly all of the carbon had been converted into carbon dioxide.
14 The antimicrobial activities of the PVA extraction film-forming solution incorporated with MN at
15 different MN concentration were analyze by disc diffusion methods against *E. coli* and *legionella*.
16 The inhibition activities were measured by the diameter of inhibition zone. The results are shown
17 in Figure 7 with the inhibition zone of the microbial in the Table III. From overall, the PVA film
18 forming incorporated with 0.75 concentration of MN (PVA_MN₇₅) inhibited excellent inhibition
19 zone of 12.17 mm. As increased concentration of extraction PVA_MN₁₀₀ films, shows least
20 effective towards *E. coli* which the inhibition zone is 10.33 mm. In other hand, same pattern of
21 results can be seen in the inhibition of PVA_MN film forming solution towards *legionella*. Where
22 the highest inhibition zone take place at 13.83 mm for PVA_MN₇₅. This is shown that PVA_MN₇₅

1 is the optimum concentration for antimicrobial activity compared to other concentration and can
2 be considered as a promising candidate for plastic packaging.

3

4 **CONCLUSION**

5 The most optimum composition of PVA and MN in making blend film was at PVA_MN₇₅, where
6 it shows improved tensile strength (2.907MPa), tensile modulus (0.113MPa), high thermal
7 degradation, moisture content (13.44 %), water solubility (12.16 %), water absorption (16.80 %)
8 with smooth and compact film morphology. The PVA_MN₇₅ film resulted in an appropriate film
9 degradation towards soil and good microbial inhibition towards *E. coli* and *Legionella* species.
10 Hence, through this study we can conclude that the mechanical properties of PVA have been
11 improved by the addition of MN as well as the water solubility properties of the films were
12 decreased. The improved properties of PVA film with the use of MN showed superior properties
13 for food packaging application. In addition, MN contributes in the crosslinking reaction and
14 exhibited good antimicrobial properties and maybe suitable candidate for plastic packaging.

15

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19

20 **Author Contributions Statement**

21 Dr. Sujan Chowdhury and Dr. Mah Shee Keat encouraged Nur Syaliani Binti Mohamed
22 Rafflisman Zaidi to investigate the overall work. Dr. Sujan Chowdhury supervised the findings of
23 this work. All authors discussed the results and contributed to the final manuscript.

24

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- 17

Figures

Figure 1

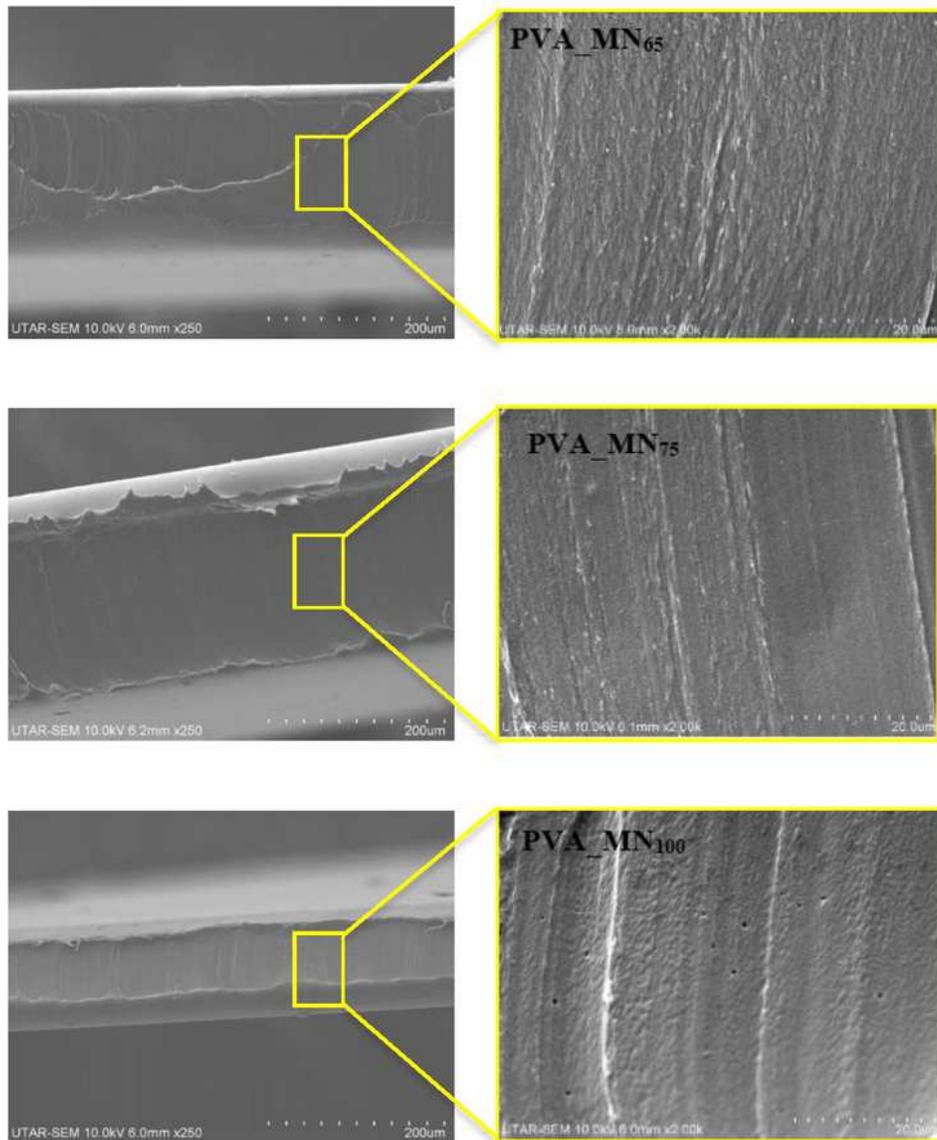


Figure 1

SEM images of the cross section of the PVA/MN blend films at different MN content.

Figure 2

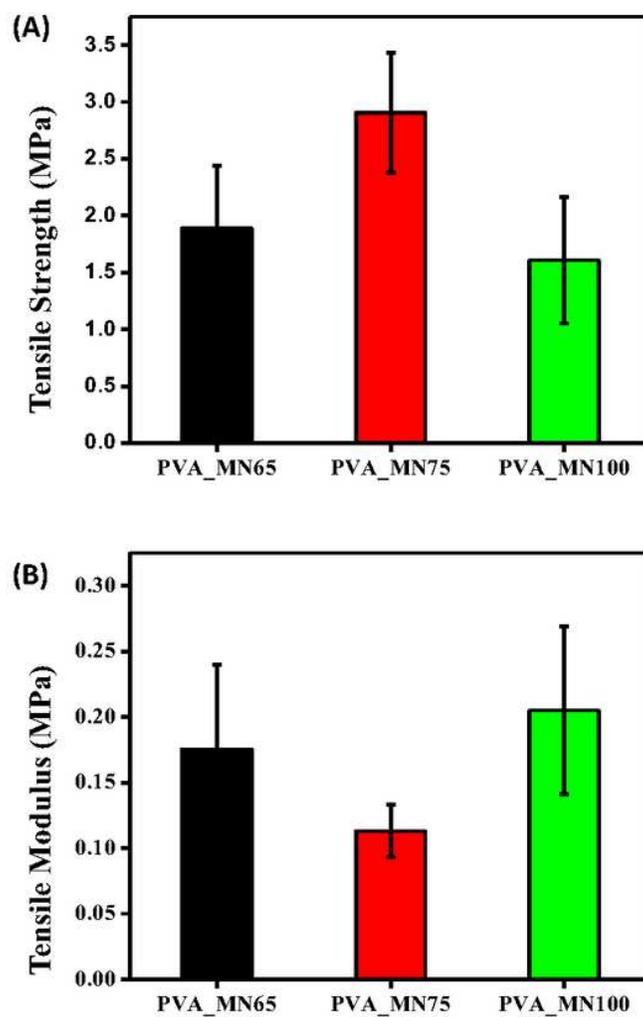


Figure 2

Mechanical properties of the PVA/MN blend films at different MN content.

Figure 3

(A) TGA and (B) DTG of the PVA/MN blend films at different MN content.

Figure 4

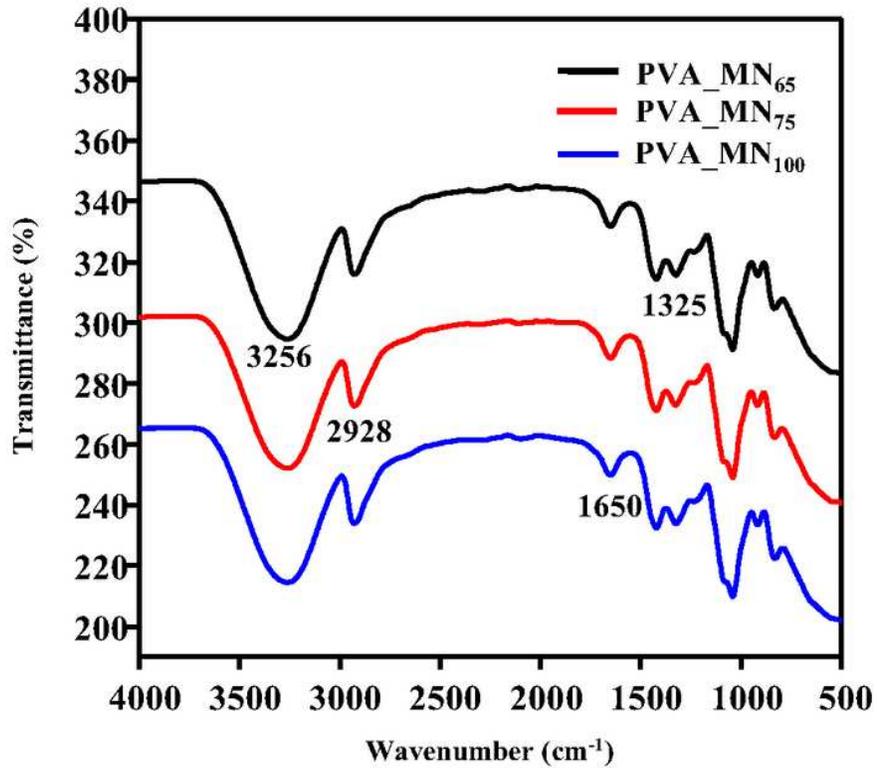


Figure 4

FTIR spectrum of the PVA/MN blend films at different MN content.

Figure 5

(A) MS; (B) WS; and (C) WA of the PVA/MN blend films at different MN content.

Figure 6

Images of the PVA/MN blend films at different MN content for biodegradability test.

Figure 7

Images of the inhibitory zone of the agar plates incubated with (a) *Escherichia coli* and (b) *Legionella* bacteria with PVA/MN film making solution at different MN content.

Supplementary Files

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