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Potential for Antibacterial Activity of Chitosan-Polyvinyl Alcohol Membrane Loaded with Green Grass Jelly Leaf and Moringa Leaf Extract as a Wound Dressing

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Abstract

Chitosan is a natural polymer that can be used as wound dressing material; however, it has rigid and brittle properties. A combination of chitosan and polyvinyl alcohol (PVA) is expected to allow improvement of chitosan's mechanical properties. Green grass jelly leaf *(Cyclea barbarta M.)* and moringa leaf (*Moringa oleifera L.*) have antibacterial compounds that can be added to the chitosan-PVA composite membrane. The purpose of the research was to develop and characterize the chitosan-PVA composite membrane with the addition of green grass jelly leaf and moringa leaf extracts to enhance the antibacterial activity of the membranes that have potential as a wound dressing. Both extracts with various composition volumes (75:25, 50:50, and 25:75) were tested for antibacterial activities against *S. aureus* and *E. coli*. Chitosan-PVA composite membrane with the volume ratios of 5:5, 6:4, and 7:3 was added with extract with the highest antibacterial activity. The composites were characterized for density, water vapor permeability, tensile strength, elongation, Fourier Transform Infrared spectroscopy, and Scanning Electron Microscope. The most significant inhibition zone was shown by an extract ratio of 50:50 against *S. aureus* and *E. coli*, 13.00±1.17 mm and 7.00±0.17 mm, respectively. Composite membrane with the addition of extract had a larger inhibition zone against *S. aureus* (9.75±0.75 mm) and *E. coli* (7.50±0.65 mm) than without extract. Chitosan-PVA(5:5)+extract membrane showed excellent density and water vapor permeability compared to other membrane ratio compositions. Mechanically, the addition of extract decreased the tensile strength and elongation of the membranes; however, it still complied with the medical material standard criteria. The characterization for functional groups showed that chitosan-PVA+extract generated the N-H group peak with two wavenumbers expressed as overlapping amides with amines and protonated amines. The SEM analysis showed that the addition of extract was not distributed homogeneously on the membrane surface.

1. Introduction

The wound dressing membrane maintains a moist environment on the wound surface, increases air exchange, acts as a barrier to the entry of microorganisms, and removes excess exudate [1]. Membranes used as wound dressings to repair the connective tissue in the injured skin are continuously being developed. One of the natural ingredients that can be developed as a wound dressing is chitosan. Chitosan is a derivative of chitin found in invertebrates such as *Crustacea* sp. [2]. Chitosan is a suitable biomaterial to be developed and applied in many fields because it is biodegradable, biocompatible, non-toxic, and hemostatic [3]. One of the roles of chitosan in the medical field is as an antibacterial agent. The antibacterial activity of chitosan is influenced by the glucosamine NH_3 group that can interact with the negatively charged bacteria

surface, which leads to interfering bacterial growth [4]. However, one of the disadvantages of chitosan is its low elasticity which causes it to be rigid; therefore, chitosan needs to be combined with other materials such as PVA to increase its elasticity.

PVA is a non-toxic, water-soluble, biocompatible polymer with excellent film-forming, emulsifying, and moisturizing properties. Due to its properties, PVA is widely used in various fields, including the medical and pharmaceutical fields [5]. According to Mutia and Eriningsih [6], wound dressing membranes can be made from alginate-PVA and a mixture of chitosan-collagen-PVA [7]. Chitosan-PVA composite membrane with the addition of pectin from green grass jelly leaf showed antibacterial inhibition against *S. aureus* and *E. coli* [8]. Sari [9] reported that the band-aid of tapioca flour-chitosan-glycerin with the addition of methanol extract of moringa leaf showed an increase in antibacterial inhibition against *S. aureus*. This shows that the addition of active ingredients to the chitosan-PVA composite membrane can increase the antibacterial activity of the membrane.

Green grass jelly leaf (*Cyclea barbata* M.) and moringa (*Moringa oleifera* L.) leaf have been widely explored because they are rich in benefits and can be used as medicine. Green grass jelly leaves are traditionally used as a febrifuge, gastric ulcer medicine, to relieve nausea, to lower high blood pressure and bacterial infections. The ethanol extract of green grass jelly leaf has antibacterial activity against *E. coli* and *Salmonella typhi*. This was evidenced by forming an inhibition zone of 17.23 mm against *E. coli* and 15.89 mm against *S. typhi* at a concentration of 100% [10]. Several components that play an active role in green grass jelly leaves, such as alkaloids, saponins, flavonoids, and phenols, are reported to have efficacy as antibacterial compounds [11]. In addition, it was reported that green grass jelly leaf extract at a concentration of 60% exhibited the healing effect of cuts on mice [12]. Moringa leaves as medicinal plants have been used as an antibacterial and treat various diseases ranging from malaria, typhoid fever, hypertension, and diabetes [13]. Moringa leaf extract has antibacterial activity against *Bacillus cereus*, *Enterococcus faecalis*, and *E. coli* with an inhibition zone of 7–9 mm at 50 mg/mL [14]. Moringa leaf ethanol extract at a concentration of 10% also affects healing burns because it has antibacterial and anti-inflammatory activity [15]. These activities occur due to the presence of secondary metabolites in moringa leaf extract, including terpenoids, flavonoids, alkaloids, steroids, tannins, saponins, and anthraquinones [16]. The addition of green grass jelly leaf and moringa leaf extracts on the chitosan-PVA composite membrane can enhance the antibacterial activity of the membrane.

In order to increase the antibacterial activity of the chitosan-PVA composite membrane, this study added a mixture of two types of plant extracts, namely green grass jelly and moringa leaf, which are known to have antibacterial activities. Therefore, this study aimed to develop and characterize the chitosan-PVA composite membrane added with green grass jelly leaf and moringa leaf extracts to enhance antibacterial activity as a wound dressing.

2. Methodology

2.1. Tools and materials

The tools used in this study were glassware, analytical balance Ohaus explore (New Jersey, United States), hot plate magnetic stirrer (Torrey Pines scientific), laminar airflow, Tecklock screw micrometer, Fourier Transform Infrared (FTIR) (Perkin Elmer Spectrum 2), Scanning Electron Microscope (SEM) (JEOL JSM-6510LA) and other supporting tools. The materials used were green grass jelly leaf, moringa leaf, ethanol 96%, chitosan with a degree of deacetylation of 87%, PVA with a molecular weight of 75,000 g/mol, distilled water. Materials for the antibacterial test were *Staphylococcus aureus* (*S. aureus*) (ATCC 25923) and *Escherichia coli* (*E. coli*) (ATCC 25922), amoxicillin, Lysogeny broth (LB) media, agar media, and other supporting materials.

2.2. Preparation of green grass jelly leaf and moringa leaf extracts

Green grass jelly leaves and moringa leaves were obtained from Blora Regency, Central Java, Indonesia. The dried leaves were selected and separated from the stems, then ground to pass through a 60-mesh sieve. The moisture content of green grass jelly leaf was 10.98%, and moringa leaf was 11.48%. Green grass jelly and moringa leaf were extracted with 96% ethanol solvent using the maceration method (2×24 hours). The ethanolic extracts of green grass jelly and moringa leaf were tested for phytochemicals based on Harborne [17] and followed by testing the antibacterial activity in duplicate on the two extracts with a composition ratio of 25:75, 50:50, and 75:25.

2.3. Synthesis of the composite membrane

The 2% chitosan solution was prepared by dissolving 2 g chitosan into 100 mL acetic acid (1%) and stirred using a magnetic stirrer until a homogeneous solution was produced. At the same time, 0.5 g PVA was dissolved into 100 mL distilled water, then heated at 70°C and stirred until homogeneous to obtain a 0.5% PVA solution.

The solutions with different 2% chitosan:0.5% PVA ratios were prepared by varying ratio compositions of 5:5, 6:4, and 7:3 and stirred using a stirrer for 60 minutes. Each solution was poured into a 20 × 20 cm mica plate and dried in an oven at 60°C for 20 hours.

Twenty-five mL of the ratio composition of green grass jelly leaf and moringa leaf extracts with the highest antibacterial activity was added to each composite membrane solution and then stirred for 60 minutes. Each mixture was poured into a 20 × 20 cm mica plate and dried in an oven at 60°C for 20 hours. The synthesis of each composite membrane was done in duplicate.

2.4. Antibacterial activity test on membranes

The synthesized composite membranes were cut into disc shapes about 6 mm in diameter and then sterilized in an ultraviolet sterilizer for 30 minutes. As much as 10% of LB media was poured into each petri dish and solidified. *S.*

aureus and *E. coli* bacteria were inoculated in the LB medium and incubated for 24 hours at 37°C. Each disc of the composite membrane was placed onto LB agar media (LBA). All of these procedures were performed aseptically and in twice. Each petri dish was incubated upside down at 37°C for 24 hours. After incubation, the inhibition zone formed was measured using a caliper [18].

2.5. Characterization of composite membranes

2.5.1. Measurement of composite membrane thickness

The dry composite membrane thickness was measured using a Tecklock screw micrometer with an accuracy of 0.01 mm. Measurements were repeated three times and taken at 13 different locations (top, bottom, and middle). The result values were expressed in mm.

2.5.2. Determination of composite membrane density

The empty pycnometer was weighed, then placed in the sample, and weighed. The pycnometer containing the composite membrane was added with distilled water and weighed. Another empty pycnometer was added with distilled water and weighed. Each weighing was recorded, and the specific gravity was calculated.

2.5.3. Determination of the water vapor permeability of the composite membrane

The petri dish was covered with aluminum foil that had been perforated with a hole area of 10% of the petri dish's surface area. A 30 mL of distilled water was put into a petri dish, and then the hole was closed with a membrane to be tested by gluing it using epoxy glue. The petri dish was pre-weighed and heated in an oven at 37°C. The sample was taken and weighed every 1 hour for 8 hours.

2.5.4. Determination of tensile strength and elongation of composite membrane

The membrane was cut into 80 mm in length and 20 mm in width. Both ends of the membrane sample were clamped onto the UTM Instron. The thickness value was measured by pressing the start button, and UTM Instron pulled the sample until it broke. The magnitude of the tensile strength and the percentage of elongation was determined by an equation. The data obtained were analyzed descriptively.

2.5.5. Determination of functional groups by FTIR

The composite membrane was dispersed with KBr followed by pressing it in a hydraulic press and then measured using FTIR. The results, including the wavenumber and percent transmittance, were then interpreted to determine the functional groups (ASTM E1252-98 method).

2.5.6. Membrane Morphological Characteristics

Surface morphology and homogeneity of the composite membrane were observed using Scanning Electron Microscopy (SEM). The composite membranes were cut to the size of specimen mount (sample container) on SEM. Subsequently, the specimen mount holder containing membranes was inserted into the specimen stage for positioning and recording images with a voltage of 20 kV and a magnification of 5,000×. All the characteristics of membrane parameters were performed in triplicate.

2.6. Data analysis

The results of the antibacterial analysis in the form of inhibition zone (mm) were presented as mean ± standard deviation (SD) and analyzed descriptively. The data of characterized membrane were expressed as mean ± standard deviation followed by a one-way analysis of variance (ANOVA) using the Minitab version with a 95% confidence interval (p = 0.05). Tukey's test for post hoc analysis was further performed if ANOVA results showed $p \leq 0.05$.

3. Results and Discussion

3.1. Extracts of green grass jelly and moringa leaves

Green grass jelly leaf and moringa leaf were extracted using the maceration method with ethanol as a solvent. The yield of green grass jelly leaf extract was 3.21%, and moringa leaf extract was slightly higher at 3.65%. This is due to the reduced water content in green grass jelly and moringa leaf during the drying process; thus, the quantity of material content is also reduced.

Phytochemical screening was conducted qualitatively to determine the presence of secondary metabolites. The green grass jelly and moringa leaf extracts showed positive results for alkaloids, flavonoids, saponins, tannins, and phenols. However, both extracts were devoid of triterpenoids. Oktavia *et al.* [19] have also reported the presence of alkaloids, flavonoids, saponins, tannins, and phenols in both extracts. Several components that play an active role in green grass jelly leaf, such as alkaloids, saponins, flavonoids, and phenols in the medical field, possess antibacterial properties [12].

An antibacterial activity test was performed to determine the potential and concentration of a compound in inhibiting the growth of microorganisms. The antibacterial activity of green grass jelly and moringa leaf extracts was tested against *S. aureus* and *E. coli* (Table 1).

The results of the antibacterial activity test of the two extracts indicated that there was inhibition of bacterial growth that may be due to the presence of secondary metabolites, such as alkaloids, flavonoids, phenols, saponins, and tannins. Among other ratios, green grass jelly-moringa leaf (50:50) produced the largest inhibition zone against *S. aureus* (13.0 mm). However, all ratios showed the same inhibition zone (7.00 mm) against *E. coli*. The antibacterial agents present in the green grass jelly-Moringa leaf (50:50) produced a synergistic effect against *S. aureus*. However, the other composition ratios were moderately ineffective in inhibiting bacterial growth because the antibacterial agents showed an antagonistic effect instead of a synergistic effect [20]. The antagonistic effect occurs because one type of antibacterial agent reduces or eliminates the effect of the other antibacterial. Davis and Stout [21] grouped the strength of the inhibition zone: weak inhibition (5 mm), moderate inhibition (5–10 mm), strong inhibition (10–

20 mm), and very strong inhibition (> 20 mm). The highest result of the antibacterial activity test was used to synthesize chitosan-PVA composite membrane added with extracts of green grass jelly and moringa leaf with a composition ratio of 50:50.

Table 1. Antibacterial activity of green grass jelly and moringa leaf extracts

Ethanolic leaf extract (96%)	mean ± SD zone of inhibition (mm)	
	S. aureus	*E. coli*
Green grass jelly leaf	12.50 ± 1.08	7.00 ± 0.17
Moringa leaf	9.00 ± 0.50	7.00 ± 0.17
Green grass jelly-moringa leaf (25:75)	11.50 ± 0.92	7.00 ± 0.17
Green grass jelly-moringa leaf (50:50)	13.00 ± 1.17	7.00 ± 0.17
Green grass jelly-moringa leaf (75:25)	11.50 ± 0.92	7.00 ± 0.17
Positive control (Amoxillin)	28.50 ± 3.75	17.00 ± 1.83

3.2. Chitosan-PVA composite membrane

The composite membrane was synthesized by varying the ratio of chitosan-PVA of 5:5, 6:4, and 7:3, respectively, with the addition of green grass jelly-moringa leaf extract (50:50) and without the addition of extract. Figure 1 shows the visual appearance of the chitosan-PVA composite membrane without and with the addition of the prepared extract.

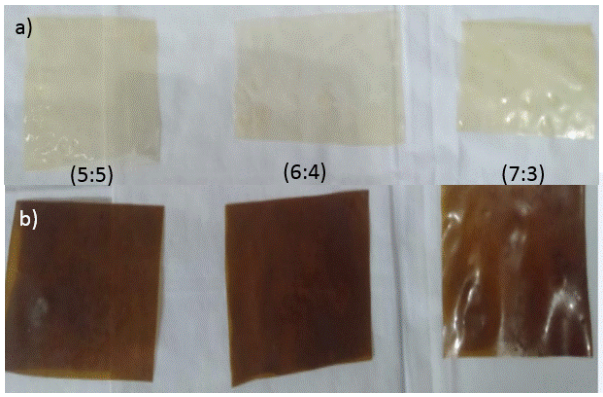

Figure 1. Chitosan-PVA composite membranes with the ratio of 5:5, 6:4, and 7:3 (a) without extract (b) with extract of green grass jelly and moringa leaf (50:50)

Figure 1 shows that the chitosan-PVA composite membranes without the addition of the extract looked transparent, whereas their color changed to brownish red with the addition of the extract. Translucent properties are influenced by the color produced on each membrane. Translucent or transparent properties can show the shape or condition of the wound, which is one of the factors needed in the development of polymer-based wound dressings [22]. The use of transparent polymers as a wound dressing is highly appropriate and in accordance with the literature. However, this is not in line with the results obtained where the addition of extract will change the transparency of the membrane.

3.3. Antibacterial activity of chitosan-PVA composite membrane

The antibacterial activity test of the membrane was conducted qualitatively on the bacterial growth of *S. aureus* and *E. coli*. The results of the antibacterial activity of the membrane against the two bacteria are presented in Table 2.

The results revealed that the chitosan-PVA membrane without the addition of extract exhibited inhibitory activity against two tested bacteria. Chitosan has an antibacterial effect due to the presence of a protonated amine group. Pan *et al.* [23] stated that the inhibitory activity against Gram-negative bacteria was due to the interaction between chitosan and negatively charged phospholipids of the bacterial cell membrane, thereby changing its permeability. This interaction allows the protein denaturation of the membrane and initiates penetration into the phospholipid layer. In addition, the increased permeability of the outer and inner membranes will cause destabilization of the cell membrane resulting in leakage of intracellular substances, which will eventually lead to the death of Gram-negative bacteria cells [24]. Inhibitory activity against Gram-positive bacteria occurred because chitosan forms a film on the surface of the bacteria, thereby preventing nutrients from entering the cells.

Table 2. Antibacterial activity of composite membranes against *S. aureus* and *E. coli*

Membrane	mean ± SD zone of inhibition (mm)					
	S. aureus			*E. coli*		
	5:5	6:4	7:3	5:5	6:4	7:3
Chitosan-PVA	6.75 ± 0.25	7.00 ± 0.00	7.50 ± 0.50	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Chitosan-PVA+extract	7.75 ± 0.25	7.75 ± 0.25	9.75 ± 0.75	6.75 ± 0.50	7.00 ± 0.35	7.65 ± 0.65

The antibacterial activity test results of the chitosan-PVA composite membrane with extract of green grass jelly and Moringa leaf had better antibacterial activity descriptively than without extract. Chitosan-PVA(7:3)+extract showed the largest inhibitory zone area, 9.75 mm for Gram-positive bacteria (*S. aureus*) and 7.65 mm for Gram-negative bacteria (*E. coli*). Gram-positive bacteria tend to be more sensitive to antibacterial components due to a simpler cell wall structure, allowing them to more easily penetrate the cells and interact with the targets to inhibit bacterial growth. In contrast, Gram-negative cells possess a more complex cell wall structure consisting of lipoproteins, lipopolysaccharides, and peptidoglycan [25].

The larger the inhibition zone diameter indicates the effectiveness of the antibacterial agent in inhibiting bacterial growth. The addition of the two extracts contributed to increasing the diameter of the inhibition zone than without one. This is because both extracts have the potential as antibacterial compounds and will increase the antibacterial activity of the membrane when added to the composite membrane. This increase in the inhibition zone indicated a synergism between the extract and chitosan. Increasing the inhibition zone of the

membrane will suppress bacterial growth, thereby accelerating wound healing. From the antibacterial activity test results, it has been proven that adding the two extracts can increase the inhibition zone of the two bacteria used in the experiment. In conclusion, it has been proven that both extracts have the potential and success as antibacterial activity.

3.4. Characterization of the composite membrane

3.4.1. Membrane thickness

The membrane thickness test aimed to determine the quality of the membrane as one of the parameters for testing the density, water vapor permeability of the membrane, tensile strength, and elongation. The thickness test was measured using a Tecklock screw micrometer with an accuracy of 0.01 mm. This measurement was taken from different sides, namely the top, middle, and bottom sides. Figure 2 shows the average value of the membrane thickness.

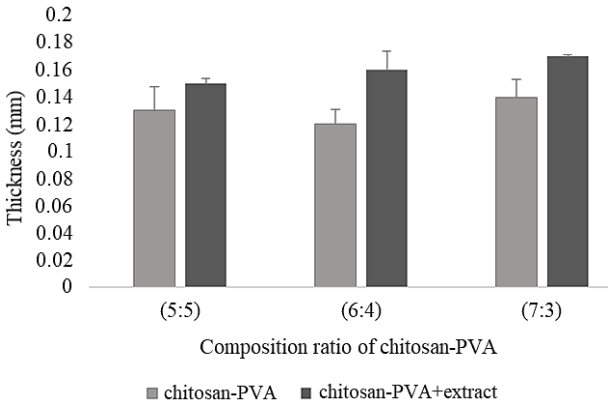

Figure 2. The thickness of chitosan-PVA composite membrane with and without extract

The addition of extract to the chitosan-PVA composite membrane led to an increase in thickness, the highest thickness of 0.17 mm was achieved by chitosan-PVA(7:3)+extract. Statistically, the thickness of the composite membrane was obtained (p = 0.00), indicating a significant difference in the thickness of the composite membrane. Tukey's test showed a significant difference in thickness between chitosan-PVA(7:3)+extract with other membrane composition ratios. The thickness of the membrane increased along with the addition of green grass jelly leaf and moringa leaf extracts. Adding extract to the membrane will result in a more concentrated solution, causing the thickness of the membrane to increase. The chitosan-PVA(7:3)+extract resulted in a larger thickness that affected higher antibacterial activity than other composite membranes. This was attributed to the large number of antibacterial compounds contained in the higher extract concentration. The thickness of the membrane can be used as quality control for wound dressing applications. An ideal wound dressing is like having a thin thickness but is not easily torn.

3.4.2. Density of membrane

This analysis was conducted to determine the strength and density of the membrane. The regularity of the membrane is proportional to the more significant the density value. The density of the chitosan-PVA composite membrane with and without the addition of extract is presented in Figure 3.

The density of the composite membrane exhibited almost significantly different results (p = 0.06). Descriptively, the chitosan-PVA composite membrane without the addition of extract had a higher density value than the membrane with the extract, attributed to the interaction between the polymer matrix—chitosan and PVA. Meanwhile, the addition of extract that fills the space between the membranes can decrease the interaction between chitosan and PVA so that low density is formed. The greater the density, the higher the tensile strength, hardness, and rigidness [26].

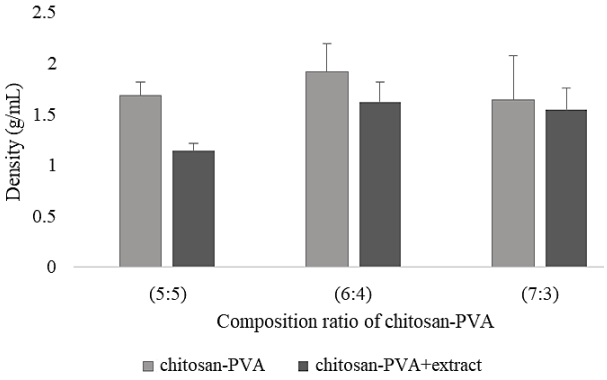

Figure 3. The density of chitosan-PVA composite membrane with and without extract

In this research, a membrane with a low rigidness is needed because a membrane with a low rigidness will be more easily stretched. Chitosan-PVA(5:5)+extract composite membrane is better than other membranes because it has the lowest density, which means that this membrane also has low rigidness. The results of this study were in accordance with previous studies [26], which showed that the addition of extract could reduce the membrane's density, leading to a decrease in rigidness.

3.4.3. Water vapor permeability of the membrane

Water vapor permeability analysis was carried out to determine the ability of the obtained membrane to absorb water vapor that penetrated the membrane. This analysis can also investigate the relationship between water vapor permeability and density. The average values of water vapor permeability are shown in Figure 4.

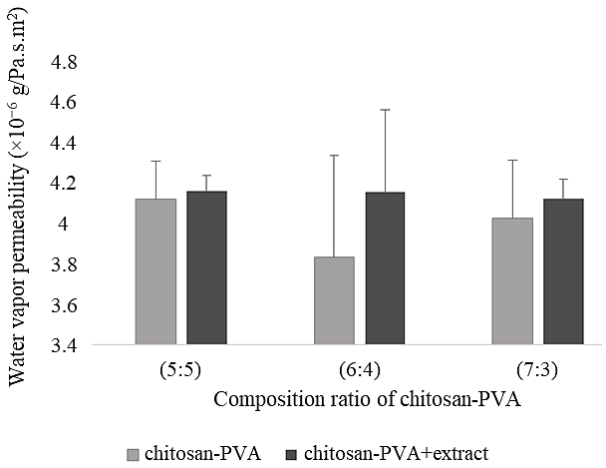

Figure 4. Water vapor permeability of chitosan-PVA composite membrane with and without extract

The water vapor permeability value showed no significant differences (p = 0.48). However, the test results showed that the highest water vapor permeability value was the chitosan-PVA composite membrane with the addition of extract. This is because the compounds in the extract have an excellent ability to bind water, which means water vapor will be absorbed, resulting in a high water vapor permeability value. The decrease in the rate of water vapor permeability with increasing density is in line with the research [27]. The low value of water vapor permeability indicated that the membrane has a high barrier capability which can protect the wound from becoming infected with more harmful microorganisms that can slow down the re-epithelialization process of the wound. The previous study [27] also presented the same results, which stated that the addition of the extract could increase the water vapor permeability and decrease the density of the membrane. The membrane has the potential to protect the wound from various microorganisms.

3.4.4. Tensile strength and elongation of membrane

The mechanical properties of the resulting composite membrane are known from the tensile strength and elongation test responses. The tensile strength value aims to determine the maximum force used to break the membrane. The results of the average tensile strength values are presented in Figure 5.

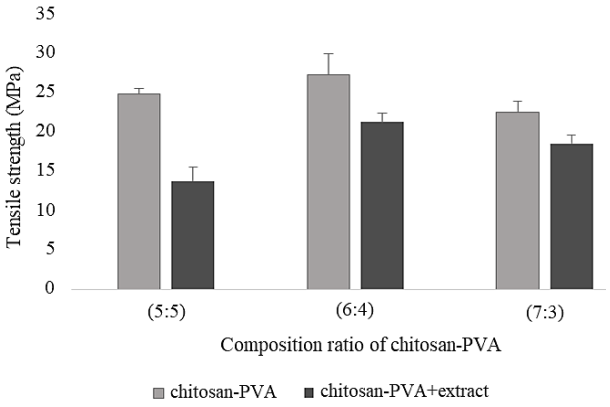

Figure 5. Tensile strength of chitosan-PVA membrane with and without extract

Chitosan-PVA(6:4) without extract showed the highest tensile strength value of 27.25 MPa. The tensile strength value has a significant difference (p = 0.00). Tukey's test revealed that the tensile strength of chitosan-PVA(5:5)+extract had a significant difference compared to other composite membrane compositions. These results indicated that chitosan could improve the membrane's mechanical properties. The physical interaction that occurs between the –OH (polyvinyl alcohol) and $-NH_2$ (chitosan) groups in the polymer composite can improve the mechanical properties of the membrane [28]. The addition of extract to the composite membrane exhibited a low tensile strength value. This is possibly linked to the small molecules of the extract filling the gap between the membranes, causing the interaction between the polymer matrix (chitosan and PVA) to decrease, which results in a reduced membrane density [29]. The tensile strength value is in line with the decrease in the membrane density.

Besides tensile strength, percent elongation can be employed to measure the membrane's flexibility. Percent elongation is the change in maximum length when stretching occurs until the sample breaks. The percentage of elongation determined the elasticity of a membrane. The higher the percentage of membrane elongation, the more elastic the membrane. The average percent elongation value can be depicted in Figure 6.

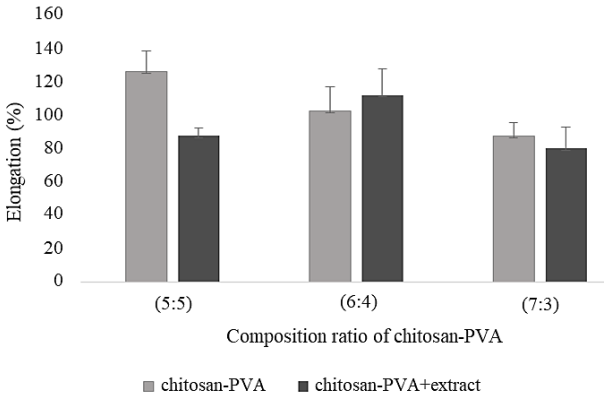

Figure 6. Elongation of chitosan-PVA composite membrane with and without extract

The percent elongation of chitosan-PVA(5:5) without extract had the highest value of 126.4%, whereas chitosan-PVA(7:3)+extract obtained the lowest percent elongation of 80.20%. The addition of extract decreased the membrane's flexibility with a significant reduction in the percent elongation of the membrane (p = 0.00). Tukey's test revealed that chitosan-PVA(5:5) without extract had the most significant difference compared to the addition of extract at the same ratio. The elongation results show a discrepancy between the results and the reference in [26] when associated with the obtained density value.

The membrane density without extract was higher than that with the extract, indicating an increase in the rigidness and tensile strength of the membrane; unfortunately, the elongation value should decrease. The elongation value should be inversely proportional to the tensile strength. However, the results of this study

showed that the membrane without extract had the highest density value, the highest tensile strength, and the highest elongation. It is proven that PVA played a role in increasing the elasticity of the membrane, but its elasticity became low when the extract was added. This indicated that the membrane with the addition of the extract had the potential to be an inhibitor of bacterial activity; however, it had little effect in terms of the mechanical properties of the resulting membrane. Perhaps this is due to the discrepancy in the comparison used.

The tensile strength and elongation values obtained have fluctuating values; this may be due to several factors, such as less homogeneous mixing that causes the insertion of the extract into the chitosan-PVA polymer matrix not to be evenly distributed. Moreover, the resulting elongation at break was less than optimal. Based on D.K.P. *et al.* [30], standard medical materials have elongation values between 17% and 207%, whereas the tensile strength values are between 1 MPa and 24 MPa. Based on these criteria, this study's tensile strength and elongation values were still included in the standard medical criteria. This showed that the chitosan-PVA composite membrane with the addition of extract is feasible as a wound dressing.

3.4.5. FTIR

FTIR spectrum analysis aimed to identify the functional groups or types of interactions contained in the resulting composite membrane. Figure 7 contains the FTIR spectra of chitosan-PVA without extract and chitosan-PVA+extract. Based on the figure, it can be seen that there is a similarity in the spectrum between the two.

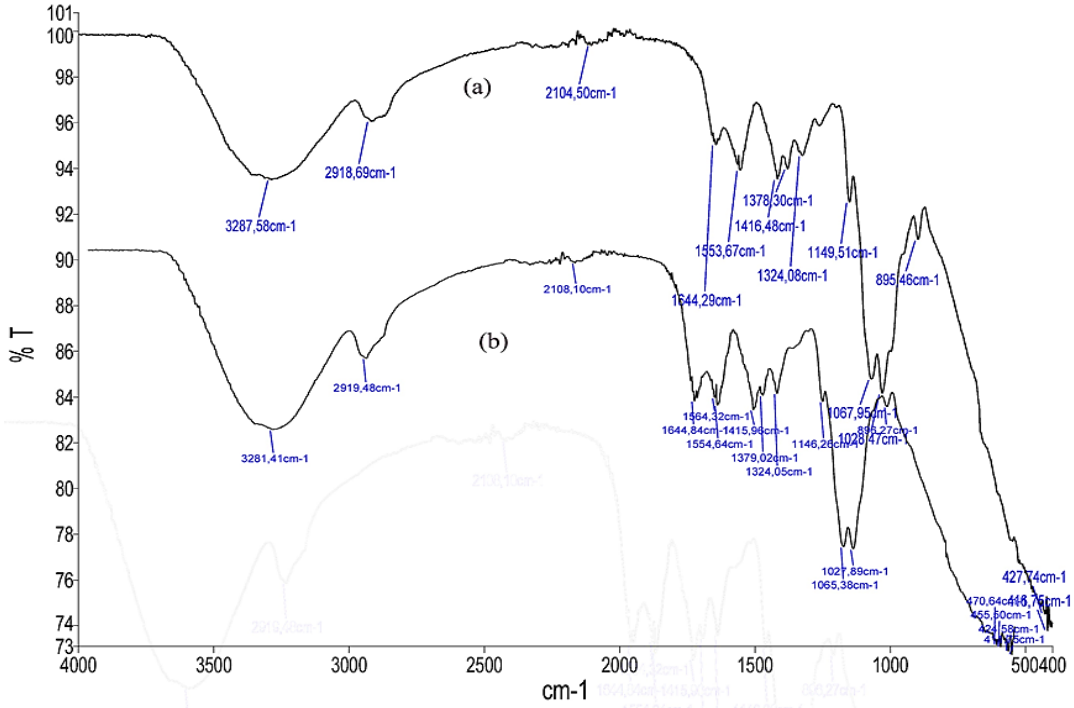

Figure 7. Comparison of the IR spectra of composite membranes (a) (chitosan-PVA) and (b) (chitosan-PVA+extract)

According to Dompeipen [31], the specific functional groups found in pure chitosan are hydroxyl groups (-OH) and amide groups (NH_2) in the infrared absorption spectrum at the range of 3200–3500 cm^{-1} and 1660–1500 cm^{-1}. Figure 7a shows the O-H absorption band at 3287 cm^{-1}, whereas the C-H absorption peak at 2918 cm^{-1}. The existence N-H group is evidenced by the absorption of the wavenumber of 1553 cm^{-1}. The stretching vibration of the C-O-C group is proven by absorption at wavenumbers 1028 and 1067 cm^{-1}. Figure 7b reveals the O-H absorption band (stretching) at 3281 cm^{-1}. The absorption peaks of the C-H (stretching) and C-O-C (bending) groups are indicated at wavenumbers 2919 cm^{-1}, 1027 and 1065 cm^{-1}, respectively. Chitosan-PVA+extract exhibits a peak of N-H group with two wavenumbers at 1564 and 1554 cm^{-1}; it was expressed as an amide overlapping with protonated amines and amines (Table 3).

The chitosan-PVA composite membrane with and without the addition of extract showed no new peaks. However, the OH group experienced a shift in wavenumber associated with cross-linking between the chitosan-PVA composite membrane and the extract. In addition, the percentage of transmittance of the O-H and C-O-C groups decreased on the chitosan-PVA composite membrane with the addition of extract.

Table 3. Results of FTIR analysis of chitosan-PVA without and with the addition of extract

Functional group	Wavenumber (cm^{-1})	
	Chitosan–PVA	Chitosan-PVA+extract
O-H	3287	3281
C-H	2919	2918
N-H	1553	1564, 1554
C-O-C	1028, 1067	1027, 1065

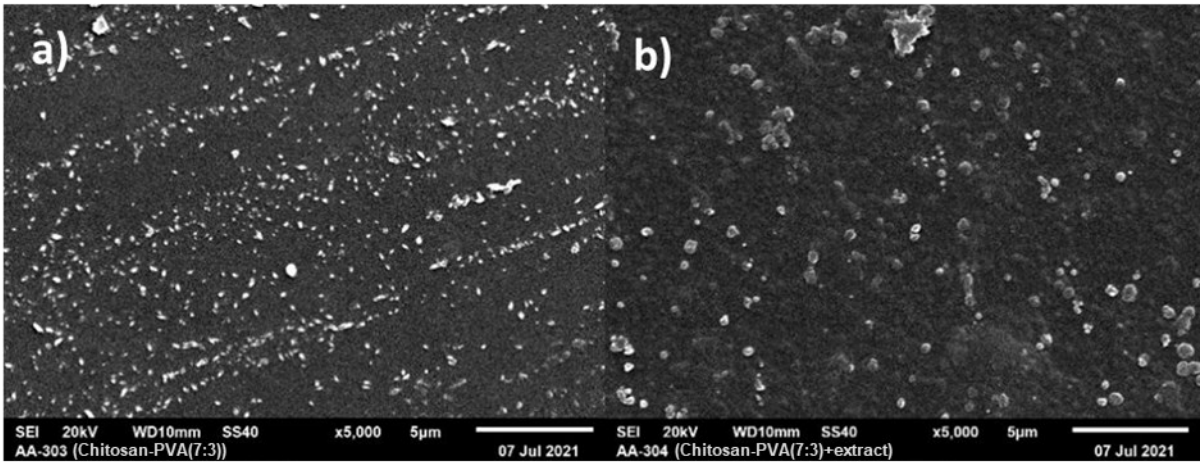

Figure 8. Morphology by SEM performed at a magnification of 5,000× (a) chitosan-PVA composite membrane (7:3) and (b) chitosan-PVA composite membrane (7:3) with extract

3.4.6. Morphology of membrane

The morphology of the membrane was measured using SEM to observe the distribution of the chitosan-PVA composite membrane with and without the addition. The resulting morphology will determine the homogeneity of the composite membrane microscopically. Morphological tests by SEM were performed on the surface of the chitosan-PVA composite membrane with a magnification of 5,000× (Figure 8). The results of the SEM analysis of the chitosan-PVA composite membrane without the addition of extract showed that there were small grains on the surface. The granules are indicated as PVA embedded in chitosan (Figure 8a).

The chitosan-PVA composite membrane with the addition of extract appeared to have an uneven distribution or not homogeneous with the accumulation of extract on the surface of the membrane (Figure 8b). The inhomogeneity was due to the uneven distribution of chitosan-PVA composite membrane without or with the addition of extract. In addition, the distribution between the chitosan-PVA composite membrane without or with the addition of extract was not completely uneven due to the stirring factor [32]. These results also affect the stability of the tensile strength and elongation measurements.

4. Conclusion

Chitosan-PVA composite membrane with the addition of green grass jelly leaf and Moringa leaf (50:50) extract can increase the antibacterial activity against *S. aureus*. Based on the density and water vapor permeability test, the chitosan and PVA composite membranes with the addition of extract were better than those without extract. Data from the density and water permeability test results revealed that chitosan-PVA with the addition of extract has the potential as a wound dressing membrane. In terms of tensile strength and elongation tests, the membrane with the addition of extract did not significantly affect the elasticity of the membrane, but the results obtained were still within the medical standard; thereby, the membranes still have potential as a wound dressing. Characterization by FTIR showed that the chitosan-PVA composite membrane with extract had amide overlapping with protonated amines and amines. Meanwhile, the SEM results showed that the distribution of the extract was not homogeneous on the composite membrane surface.

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