

Addition of black pepper extract as an antibacterial in the manufacture of polyvinyl alcohol (PVA) and carboxymethyl cellulose (CMC) based hydrogel wound dressings

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ARTICLE INFO

Article history:

Received Oct 18, 2025

Revised Oct 23, 2025

Accepted Oct 30, 2025

Keywords:

Antibacterial
Citric Acid
Black Pepper Extract
Wound Dressing
PVA/CMC

ABSTRACT

This study aims to determine the effect of adding black pepper (*Piper nigrum* L.) extract on the characteristics of a hydrogel wound dressing made from a mixture of Polyvinyl Alcohol (PVA) and Carboxymethyl Cellulose (CMC), and citric acid as a crosslinking agent. The manufacturing process was carried out by chemical crosslinking with varying concentrations of CMC (5%, 7.5%, 10% w/v) and black pepper extract (0%, 10%, 20%, 30%, 40% v/v). The analysis included FTIR, SEM, swelling degree, water solubility, tensile strength, and antibacterial activity against *Staphylococcus aureus*. The results showed that the addition of black pepper extract increased antibacterial activity and water absorption (swelling) up to 200-500%, in accordance with the standards for good wound dressings. However, increasing the concentration of black pepper extract tended to decrease water solubility due to its hydrophobic nature. Overall, the PVA/CMC hydrogel with the addition of black pepper extract showed potential as a biocompatible wound dressing with good mechanical properties and antibacterial activity. The gel released 23.12 μ g/cm²curcumin within 150 minutes.

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INTRODUCTION

A wound is damage to body tissue caused by factors that disrupt the body's protective system (Paksi, Suryati, Muarif, ZA, & Meriatna, 2023) Wounds are often difficult to treat, especially if they have been invaded by bacteria. Bacterial infections can seriously delay the wound healing process. In some cases, wound healing often goes awry, leading to pathological problems such as hypertrophic scars and keloids (Pratiwi & Siahaan, 2023).

Hydrogel wound dressings are three-dimensional hydrophilic materials that can absorb large amounts of water and biological fluids, up to 99% of them. Hydrogel wound dressings have lower oxygen permeability, resulting in higher oxygen pressure outside the wound surface. This results in faster natural collagen formation in the surrounding tissue (Tarmizi, Maharsih, Jannah, &

Wahyuni, 2020). Currently, various advances have been achieved in the wound healing process using biocompatible and biodegradable polymers with antimicrobial properties that can provide optimal wound healing. (Fahmi, 2020).

The use of hydrophilic polymers such as polyvinyl alcohol (PVA) as biomaterials has attracted considerable attention due to its non-toxic, non-carcinogenic, and high biocompatibility. However, this polymer has the mechanical properties of PVA hydrogels, which are easily brittle (Mahsunah, 2015). Therefore, it needs to be modified by combining it with synthetic or natural polymers that not only function to increase its mechanical properties, but can also accelerate the healing of wounds on the skin. (Haryanto & Selviana, 2022) However, PVA does not have antimicrobial activity. (Mata, Morais, Oliveira, & Aguiar, 2022), then it is necessary to add a biocidal agent.

Carboxymethyl cellulose (CMC) polymer is a water-soluble, non-toxic, relatively inexpensive, and biodegradable material with excellent qualities in the formation of hydrogel films (Arefian et al., 2020) CMC has a high capacity to absorb exudate and maintain optimal conditions. Furthermore, CMC can activate macrophages and increase cytokine levels in wounds (Kanikireddy, Varaprasad, Jayaramudu, Karthikeyan, & Sadiku, 2020). In several previous studies, it was found that the addition of CMC affected the swelling and mechanical properties of hydrogel wound dressings.

Pepper is one of the plants that has been shown to have antibacterial activity. The chemical constituents of pepper seeds include alkaloids, phenols, anthraquinones, tannins, coumarins, saponins, flavonoids, glycosides, and essential oils. Pepper seed extract contains 5-9% of the main compounds piperine, piperidine, piperethine, and piperine. Piperine is the major alkaloid in pepper seeds and has been shown to have antibacterial activity against *Staphylococcus aureus* (Hasriyani, Zulfa, Anggun, & Murhayati, 2020). In addition, black pepper also has very strong activity as an anti-termite.

Gum arabic is used as a binder because it has the characteristics of emulsification, non-toxicity, commercial availability, biocompatibility, and good film formation, gum arabic can also overcome stability, solubility and bioavailability problems, and antioxidants due to the presence of amino acids. (Amalraj, Haponiuk, Thomas, & Gopi, 2020).

In this study, hydrogel wound dressings based on PVA/CMC and black pepper extract are expected to produce wound dressing products that are antibacterial, have high water absorption capacity and have good mechanical properties.

RESEARCH METHOD

Material

The materials used in this study were polyvinyl alcohol (99.6% mol hydrolyzed), carboxymethyl cellulose (Na-CMC 0.5%), citric acid monohydrate from Sigma Aldrich, USA. Black pepper extract from Our Garden Indonesia store, deionized water from a local chemical store, and gum arabic from Mitra Jaya Chemical store.

Preparation of Materials and Making Hydrogen

The hydrogel preparation was initiated with the preparation of 15% (w/v) PVA solution, 5%, 7.5%, 10% (w/v) CMC solution and 10% GA solution. PVA solution (15% w/v) was made by dissolving 15 grams of PVA powder in 100 ml of deionized water and homogenized using a magnetic stirrer at a temperature of 87°C. Preparation of CMC solution (5%, 7.5% and 10% w/v) was carried out by dissolving 5, 7.5 and 10 grams of CMC powder in 100 ml of deionized water with constant stirring at a temperature of 60°C until the solution was homogeneous. Preparation of GA (10% w/v) was made by dissolving 10 grams of PVA powder in 100 ml of deionized water and homogenized using a magnetic stirrer at a temperature of 50°C. Next, a PVA/CMC/GA solution was prepared in a beaker, followed by citric acid and glycerol. The PVA/CMC/GA solution was then combined with black pepper extract in varying concentrations. The formed hydrogel was poured into a petri dish and dried in an oven at 50°C.

Antibacterial Testing

Antibacterial activity is developed to prevent the growth of pathogenic microorganisms such as bacteria. (Siregar, Ardiansyah, & Asna, 2023) This analysis was conducted in the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) at Sriwijaya University. This antibacterial analysis was conducted using the disc diffusion test method by measuring the diameter of the clear zone. The inhibition zone calculation formula can be used as follows:

$$\text{Inhibition zone diameter} = \frac{(Dv - Dc) + (Dh - Dc)}{2} \quad (1)$$

Where Dv: Vertical Diameter, Dh: Horizontal Diameter and Dc: Disc Diameter.

Swelling Degree and Water Solubility

Swelling Degree analysis is an important parameter to determine the properties of hydrogel wound dressings in absorbing water. Meanwhile, Water Solubility analysis is a key factor in hydrogel wound dressings, which is an indicator to evaluate the stability of the film in terms of water resistance, because the solubility of the film depends on the type and concentration of components as well as their hydrophilic and hydrophobicity indices. (Tasyrah A & Justitia K, 2024). The swelling degree and Water Solubility analysis was carried out by cutting the sample with a size (1 x 2 cm), then measuring the initial weight of the hydrogel sample (W1), then the sample was immersed in 30 ml of distilled water and stirred at a speed of 30 rpm at room temperature (28± 2oC) for 15 minutes and after that the soaking was left for 24 hours. Then the weight of the sample was measured after soaking (W2). The swelling degree of this wound dressing can be calculated using the equation:

$$\% \text{ Swelling Degree} = \frac{W_2 - W_1}{W_1} \times 100 \% \quad (2)$$

Where, W2 is the weight of the wet hydrogel at time t, and W1 is the initial weight of the hydrogel sample before soaking.

Next, the sample was dried at 50oC for 24 hours, then reweighed to obtain the Water Solubility calculation (Akyuz et al., 2018). Water Solubility can be calculated using the following equation.

$$\% \text{ Water Solubility} = \frac{(W_1 - W_3)}{W_3} \times 100 \% \quad (3)$$

Where W3 is the final weight after drying.

Tensile Strength and Elongation Analysis

Tensile strength analysis is a parameter to determine the resistance of a material to the tensile force applied, while elongation analysis is a parameter that shows more about the elastic properties of hydrogel wound dressings. This analysis was conducted at the Sriwijaya State Polytechnic Laboratory (POLSRI), by adapting the JIS K7162-2 (DIN EN ISO 527-2) method (ISO 527-2). Hydrogel wound dressing samples were cut to a size of 10 cm x 2 cm with a thickness of 3 mm. Then the data obtained will be processed into tensile strength (σ) with the following equation:

$$\sigma = \frac{F}{A} \quad (4)$$

Where σ is Tensile Stress (N/mm²) → (1N/cm²=1Mpa= 103KPa), F is Maximum Stress (N), and A is Cross-Section Area (m²)

Meanwhile, the Extension Analysis can be calculated using the following equation:

$$\varepsilon = \frac{l - l_0}{l_0} \times 100 \% \quad (5)$$

Where ε is the elongation of the material (m), l_0 is the initial length of the material and l is the length of the material after being subjected to stress.force (m).

Fourier Transformed Infrared (FTIR)

FTIR is an instrument used to detect functional groups, analyze a mixture of samples to be analyzed without damaging the sample, and to determine the compounds contained, such as organic and inorganic compounds, in the sample to be analyzed. FTIR analysis of this hydrogel wound dressing was conducted at the Bogor Rubber Research Center Laboratory, on the best sample, where the sample was formed with a size of 1 x 1 cm.

Scanning Electron Microscope (SEM)

A scanning electron microscope is a microscope that uses electrons as light to view objects with high resolution. A scanning electron microscope works by using electron waves emitted by an electron gun, which are then condensed on the condenser lens and focused as a clear point by the objective (Gunawan & Azhari, 2010). This SEM analysis was performed on the best samples and directly observed the morphology of the solid object's surface under magnification. The equipment used was an Axia ChemiSEM.

RESULTS AND DISCUSSIONS

The Effect of Adding Black Pepper Extract as an Antibacterial on the Characterization of PVA/CMC-Based Hydrogel Wound Dressings

PVA/CMC-based hydrogel wound dressings for wound healing applications must have antibacterial activity as a standard for good and optimal wound dressings. Therefore, black pepper extract is needed as an antibacterial agent. Black pepper extract has been shown to have antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*. (Rosa et al., 2018) In this study, the bacteria used were *Staphylococcus aureus*. Antibacterial activity analysis was conducted using the disc diffusion test method by measuring the diameter of the clear zone.

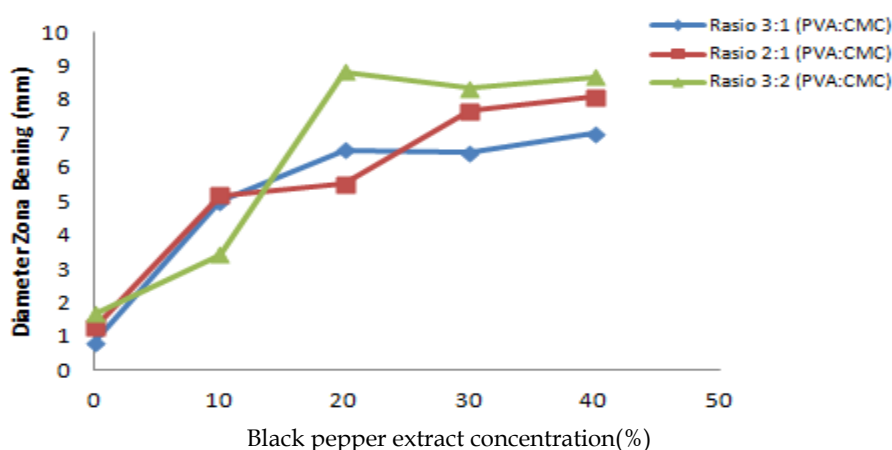


Figure 1. The effect of black pepper extract on antibacterial

Figure 1 shows that black pepper extract as an antibacterial in hydrogel wound dressings has the ability to inhibit *Staphylococcus Aureus* bacteria. This is indicated by the average range of clear zone diameters produced of 0.83 - 8.83 mm. The clear zone diameter in the 3:2 variation hydrogel wound dressing sample with the addition of 20% black pepper extract has the largest clear zone diameter of 8.83 mm. Meanwhile, the hydrogel wound dressing sample with the smallest clear zone diameter was produced in the 3:1 variation without pepper extract, which had a clear zone of 0.83 mm. The resulting clear zone diameter increased on average with increasing black pepper extract. However, there were some clear zone diameters that fluctuated (rising and falling). This is likely due to the black pepper extract not being evenly bound to the hydrogel, due to the high hydrophobic nature of black pepper extract. (Sari, Dahlan, & Susmanto, nd).

The Effect of Adding Black Pepper Extract Concentration on the Percentage of Swelling Degree and Water Solubility of PVA/CMC-Based Hydrogel Wound Dressings.

Swelling Degree analysis is an important parameter to determine the properties of hydrogel wound dressings in absorbing water. (Chopra et al., 2022). The Swelling Degree percentage can indicate how much the solution can diffuse into the polymer network and swell and be trapped. Meanwhile, Water Solubility analysis is the main factor in hydrogel wound dressings, which is an indicator to evaluate the stability of the film in water resistance, because the solubility of the film depends on the type and concentration of components as well as the

hydrophilic and hydrophobicity indices. The results of the Swelling Degree and Water Solubility analysis can be seen in Figures 2 and 3.

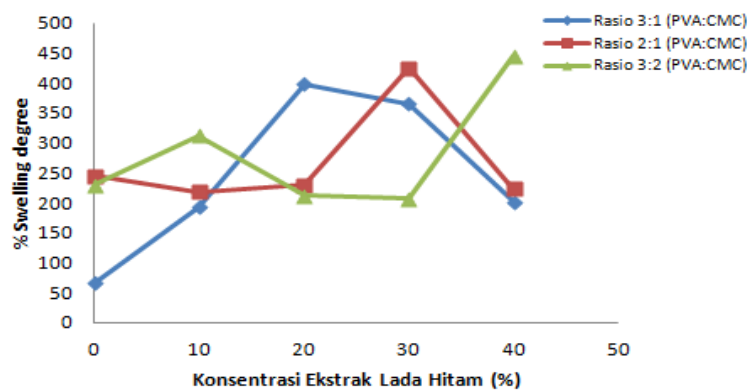


Figure 2. The effect of black pepper extract on % swelling degree

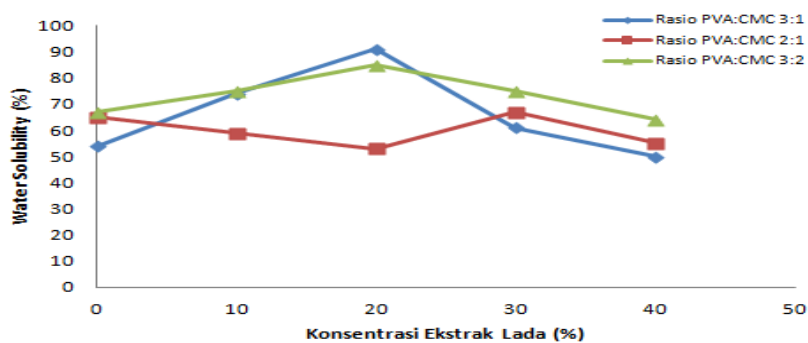


Figure 3. The effect of black pepper extract on water solubility

Figures 2 and 3 show that the percentage of swelling degree and the percentage of water solubility of PVA/CMC hydrogel with the addition of GA, black pepper extract and citric acid, experienced fluctuations in each variation of black pepper extract. The highest percentage value of swelling degree was in a ratio of 3:1 with the addition of 20% pepper extract obtained a value of 399%. In a ratio of 2:1 with the addition of 30% pepper extract obtained a value of 425%, and in a ratio of 3:2 with the addition of 40% black pepper extract obtained a value of 446%. Meanwhile, the lowest percentage value of swelling degree was in a ratio of 3:1 without pepper extract obtained a value of 65%. In a ratio of 2:1 with the addition of 20% black pepper extract obtained a value of 229% and in a ratio of 3:2 with the addition of 30% pepper extract obtained a value of 207%.

Meanwhile, the water solubility percentage value is the same as the percentage value of the sewing degree, which fluctuates with each variation of black pepper extract. The highest value is at a ratio of 3:1 with 20% black pepper extract at 54% and the lowest is at 50% with the addition of 40% pepper extract. At a ratio of 2:1, the highest is 71% with the addition of 10% pepper extract and the lowest is 55% with the addition of 40% pepper extract. Meanwhile, at a ratio of 3:1, the highest is at 85% with 20% black pepper extract and the lowest is 64% with the addition of 40% black pepper extract. (Saarai, Kasparkova, Sedlacek, & Saha, 2011).

Based on research conducted by (Rahmadani, Budiyo, & Suhartono, 2017), explained that the standard swelling degree value for wound dressings designated as wound dressings is 200-500%. Therefore, in this study, the swelling degree percentage obtained is in accordance with the established standard. Meanwhile, the water solubility value in the study conducted by Amalraj et al. (2020) and (Kavoosi, Shakiba, Ghorbani, Dadfar, & Purfard, 2015) Water solubility results of 30% and 48% have been found, so this water solubility value is considered good for wound dressing applications.

The Effect of Adding Black Pepper Extract Concentration on the Mechanical Properties of PVA/CMC-Based Hydrogels.

This analysis is one of the very important parameters to determine the physical properties and durability of hydrogel wound dressings. (Tarmidzi et al., 2020) This analysis consists of tensile strength and elongation. Tensile strength analysis is a parameter to determine a material's resistance to applied tensile forces, while elongation analysis is a parameter that more closely reflects the elastic properties of the hydrogel wound dressing. The tensile strength and elongation results can be seen in Figures 4 and 5.

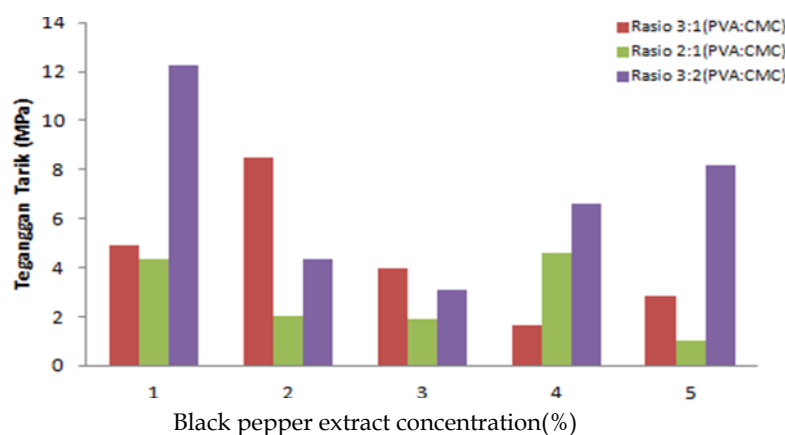


Figure 4. The tensile strength value of PVA/CMC hydrogel with

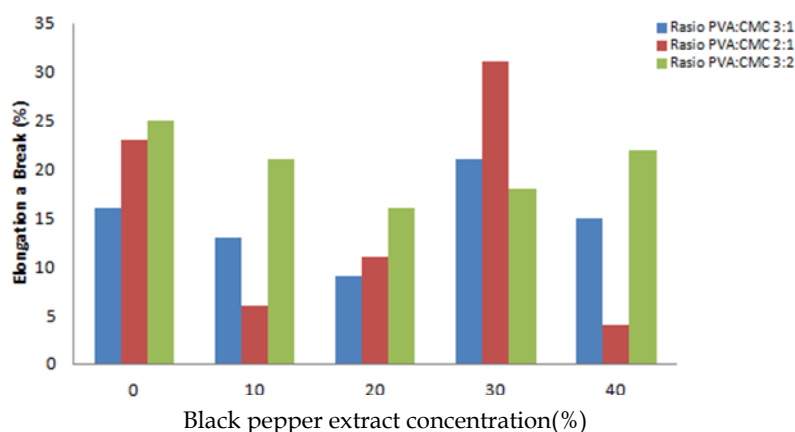


Figure 5. % Elongation a Break value of PVA/CMC hydrogel with addition of black pepper extract

Figures 4 and 5 show that the addition of black pepper extract to the PVA:CMC variation causes the TS value to fluctuate. In the 3:1 variation, the highest TS value is found in the addition of 20% pepper extract, which is 8.4894 MPa. The 3:2 variation is found in the addition of 30% pepper extract, which is 4.5984 MPa and the 2:1 variation is found without the addition of pepper extract, which is 12.2625 MPa. Meanwhile, the lowest value is in the 3:1 variation, which is 1.6350 MPa with 30% pepper extract. The 3:2 variation is 1.0219 MPa with the addition of 40% pepper extract and the 2:1 variation is 3.0656 MPa with the addition of 20% pepper extract. This is due to the addition of citric acid and pepper extract.

Meanwhile, the % Elongation a Break value of the PVA:CMC hydrogel with the addition of black pepper extract concentration and the addition of citric acid as a crosslinking agent. The % Elongation a Break value also experienced fluctuations, similar to the tensile strength value. The highest value was in the 3:1 sample, which was 21% with the addition of 30% pepper extract. In the 3:2 sample, it was 31% with the addition of 30% pepper extract, and in the 2:1 sample, it was 25% without pepper extract. (Imani, Wariyah, & Yulianto, 2025). Meanwhile, the lowest value in the 3:1

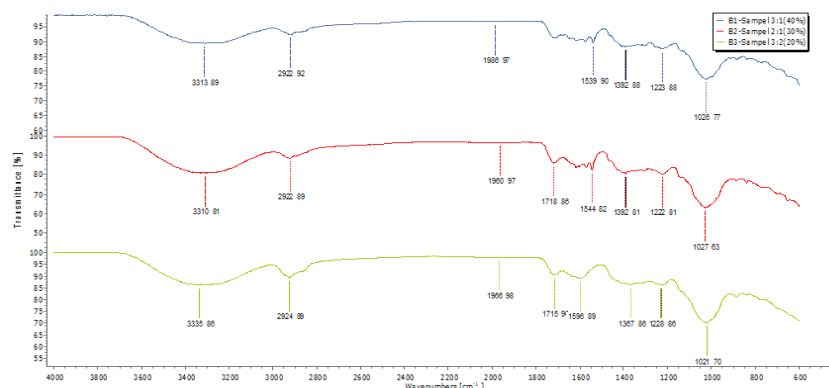


Figure 7. Spectrophotometry of PVA/CMC Hydrogel wound dressings 3:1(B1 40% pepper extract), 2:1(B2 30% pepper extract), and 3:2 (B3 20% pepper extract)

In Figure 7, with samples of 3:1 (40% black pepper extract), 2:1 (30% black pepper extract) and 3:2 (20% black pepper extract). The results obtained also show wave numbers of 3314 cm^{-1} , 3311 cm^{-1} , and 3336 cm^{-1} , these waves are hydroxyl functional groups (OH), these functional groups are caused by the presence of intramolecular hydrogen bonds. At wave numbers of 2923 cm^{-1} and 2925 cm^{-1} are saturated aliphatic CH functional groups derived from the cellulose chain. At wave numbers of 1987 cm^{-1} , 1961 cm^{-1} , and 1967 cm^{-1} , functional groups ($\text{CH}_2=\text{C}=\text{CH}_2$ stretching) were found. Wave numbers of 1719 cm^{-1} , 1716 cm^{-1} are C=O stretching vibration functional groups, which are caused by the formation of esters as a result of cross-linking. The wave numbers 1599 cm^{-1} , 1545 cm^{-1} and 1597 cm^{-1} are the COO^- functional group, which is formed due to the binding of the carbonyl group to the hydroxyl functional group. (Shivakumara & Demappa, 2019). Wave numbers 1393, and 1368 are CH functional groups. Wave numbers 1224 cm^{-1} , 1223 cm^{-1} , 1229 cm^{-1} , 1027 cm^{-1} , 1028 cm^{-1} , and 1022 cm^{-1} are CO. Based on the FTIR results obtained from PVA/CMC-based hydrogel wound dressings with a combination of GA and black pepper extract, functional groups ($\text{CH}_2=\text{C}=\text{CH}_2$ stretching) were found at wave numbers 1987 cm^{-1} , 1961 cm^{-1} , and 1967 cm^{-1} , the formation of these functional groups due to the interaction between synthetic polymers, natural polymers and black pepper extract.

Scanning Electron Microscope (SEM) Analysis

This SEM analysis was conducted on the best samples, namely samples 3:2 (0% without pepper extract) and 3:2 (20%), in this analysis, morphological observations were made from the surface of the solid object directly with magnification. Analysis of the hydrogel wound dressing samples was carried out with three variations, namely 5 μm , 10 μm , and 50 μm . This analysis was carried out at the Laboratory of the Faculty of Engineering Testing Center (LPPFT) Sriwijaya University, Indralaya Campus, Palembang. Figures 8 and 9 show the morphological results.

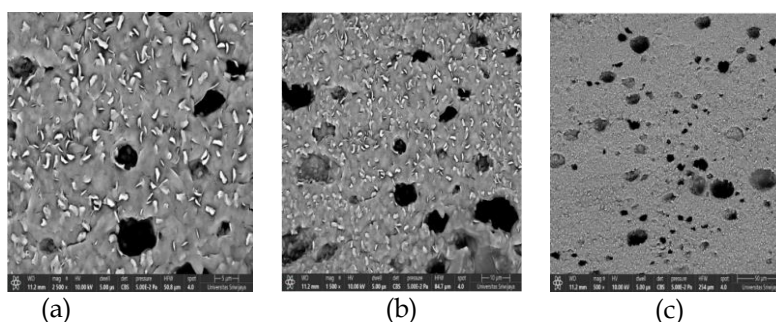


Figure 8. SEM analysis results of hydrogel wound dressing sample 3:2 (0%) with various magnifications: (a) 5 μm ; (b) 10 μm ; and (c) 50 μm

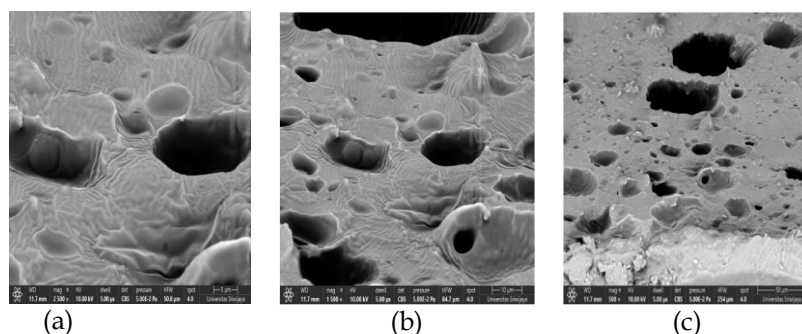


Figure 9. SEM analysis results of hydrogel wound dressing sample 3:2 (20%) with magnification variations: (a) 5µm; (b) 10µm; and (c) 50µm.

From figure 8 shows that the hydrogel film without the addition of black pepper extract, has a less porous surface morphology, less smooth, polymer particles clump together, and are denser, this is due to a higher degree of formation, so that it can limit the water absorption capacity. While in figure 9 shows that the hydrogel film with the addition of black pepper extract, shows a heterostructured hydrogel film morphology, this is because the black pepper extract is trapped in the hydrogel film network, thus increasing roughness and more pores, larger pores, without cracking due to the hydrophobicity of the black pepper extract in the hydrogel.

CONCLUSION

Based on the main objective of the study, it can be concluded that the addition of black pepper extract to PVA/CMC-based hydrogel wound dressings can increase antibacterial activity against *Staphylococcus aureus* bacteria, where the higher the concentration of the extract used, the greater the inhibitory power. In addition, variations in CMC concentration affect the physical characteristics of the hydrogel, such as increasing the swelling degree value and mechanical properties due to the formation of stronger hydrogen bonds between polymers, while its effect on water solubility is fluctuating. Overall, PVA/CMC hydrogel wound dressings with the addition of black pepper extract show good potential as a wound healing material that has antibacterial properties, water absorption, and mechanical strength that meets medical application standards.

ACKNOWLEDGEMENTS

The author's gratitude goes to the relevant parties who assisted in this research and also goes to the Sriwijaya University Research Institute which has funded this research through the DIPA Budget of the Sriwijaya University Public Service Agency for the 2024 Fiscal Year.

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