

# Synthesis of Antibacterial Coating Film Based on Eugenol-Allyl Eugenol Copolymer with Chitosan-Gelatin

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## Abstract

The development of coating film materials based on biopolymers and active antibacterial compounds has attracted attention in the food industries. Food packaging biopolymers can be increased antibacterial properties by adding compound modification of natural ingredients such as eugenol-allyl eugenol copolymer (PEAE). The aims of this study were to synthesize a coating film based on chitosan-gelatin with PEAE and test its antibacterial properties. PEAE synthesis was carried out by polymerization reaction with the  $(\text{BF}_3\text{O}(\text{C}_2\text{H}_5)_2)$  as catalyst and characterized by FTIR, molecular weight, and solubility. Synthesis of chitosan-gelatin coating films with variations in PEAE concentration of 1.25%, 2.5%, and 3.75% and characterization includes FTIR, SEM, TS, E%, and contact angle. Antibacterial activity is carried out by the turbidimetry method. PEAE was synthesized with the results in the form of brown solids with 94.91% yield, molecular weight of 9,553.98 da, and the melting point of 95-98 °C. Chitosan-Gelatin films with the addition of PEAE produce a thin yellowish film, with a sequential decreased tensile strength, and the percentage value of extension increases with the increase in PEAE concentration. The best antibacterial activity in the film PEAE 2.5, with the percentage of inhibition of *Staphylococcus aureus* and *Escherichia coli* of 99.71% and 98.39% respectively.

**Keywords:** Antibacterial, chitosan-gelatin, coating film, copolymer, eugenol-allyl eugenol.

## 1. INTRODUCTION

Researchers are increasingly focused on finding new ways to control harmful microbes across key sectors like healthcare, food production, water treatment, and textiles. One promising approach is the development of innovative antibacterial coating films. These ultra-thin films create a protective barrier on surfaces, helping to shield them from bacterial contamination. With their ability to effectively inhibit the growth of harmful microorganisms, these coating films hold great potential for widespread use in industrial applications<sup>1</sup>. Coating films as food packaging materials are crucial in extending shelf life and protecting against spoilage from various factors, including water vapor permeability, gas barriers, light transparency, microbial activity, and humidity<sup>2</sup>. In medical applications, antibacterial coating films can accelerate wound healing by preventing bacterial infections and stimulating tissue regeneration<sup>3</sup>. Coating films can be made from biodegradable

biopolymers such as proteins, polysaccharides, and lipids, with several examples of commonly used biopolymer components including chitosan, alginate, starch, gelatin, pectin, carrageenan, and xanthan gum<sup>4</sup>.

Chitosan and gelatin biopolymers are commonly used as the basis for producing high-quality films<sup>5,6</sup>. Chitosan's ability to protonate amino groups at pH below 6.5, its excellent antibacterial activity due to the presence of amino polysaccharide groups, and its biodegradable nature make it a popular choice for coating film production<sup>7,8</sup>. However, chitosan has poor mechanical properties and water barrier performance<sup>9</sup>. Therefore, gelatin is chosen to address these issues when mixed with chitosan. Gelatin is typically derived from various animals (fish, pigs, cows, etc)<sup>10</sup>. Gelatin has flexible characteristics due to its high content of proline, glycine, and hydroxyproline<sup>11</sup>. Additionally, gelatin has superior characteristics as a coating film material, such as being edible, biodegradable, having good barrier properties,

and being transparent<sup>12</sup>. The chitosan and gelatin composite can form a polyelectrolyte complex, resulting in a coating film with excellent physical properties<sup>13</sup>.

In the production of coating films, functional additives are required to achieve better results by enhancing their mechanical properties and antimicrobial activity<sup>14</sup>. It is done to reduce microbial growth on the substrate by using antimicrobial agents, such as essential oils<sup>15</sup>. One of the major components in essential oils is eugenol, which possesses strong antibacterial properties<sup>16</sup>. The hydroxyl group (-OH) in eugenol makes it a functional monomer that is reactive against organisms such as *E. coli*, *S. aureus*, and *P. aeruginosa*<sup>17</sup>. However, several limitations restrict its use in various applications, including its volatile nature, liquid form, weak mechanical properties, and low thermal stability<sup>16</sup>. To overcome these limitations, copolymerization combines eugenol with other monomers, resulting in a polymer with enhanced properties<sup>18</sup>.

Cross-linking agents play a crucial role in the copolymerization of eugenol because they affect the properties of the synthesized copolymer, thermal stability, mechanical strength, and hydrophobicity. Allyl eugenol, which acts as a cross-linking agent, is expected to cross-link with two allyl groups on eugenol, forming an eugenol-allyl eugenol copolymer (PEAE)<sup>19</sup>. This reaction occurs through cationic addition polymerization using boron trifluoride diethyl etherate (BF<sub>3</sub>O(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>) as a catalyst, which acts as a Lewis acid<sup>20</sup>.

The objective of this research was to contribute to efforts to enhance the utility of natural materials that have the potential for industrial-scale applications, such as an alternative antibacterial coating film based on eugenol-allyl eugenol copolymer (PEAE) with chitosan-gelatin. The study employs variations in PEAE concentration during the synthesis of the coating film to determine the best antibacterial activity. The characterization of the coating film includes the analysis of functional groups, morphological structure, mechanical properties, and antibacterial activity testing.

## 2. RESEARCH METHODS

### 2.1 Materials and Instrumentation

The materials used include eugenol (C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>, Sigma Aldrich), allyl eugenol, distilled water, nitrogen gas (N<sub>2</sub>), chloroform (CH<sub>3</sub>Cl, Meck, pro analysis), boron trifluoride diethyl etherate (BF<sub>3</sub>O(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>, Merck), methanol (CH<sub>3</sub>OH, Merck, pro analysis), anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>, Merck), chitosan (CV. Multiguna), glacial acetic acid/GAA (CH<sub>3</sub>COOH, Merck), gelatin, glycerol, dimethyl sulfoxide ((CH<sub>3</sub>)<sub>2</sub>SO, Merck), NA (Nutrient Agar), NB

(Nutrient Broth), *S. aureus* bacterial stock, *E. coli* bacterial stock, chloramphenicol, gauze, cotton, mattress ties, aluminum foil, plastic wrap, universal pH paper, and filter paper. The equipment and instruments used include glassware, an oven, an Ubbelohde viscometer, an FTIR Agilent Cary 630, and a Hitachi SU3900 SEM.

### 2.2 Synthesis of PEAE

PEAE synthesis was carried out by following the procedures carried out by Ngadiwiyanana et al. (2022) with slight modifications. Eugenol (0.0914 mol, 14.15 mL) was added to a three-neck flask and purged with nitrogen gas (N<sub>2</sub>) at room temperature. Allyl eugenol (0.00914 mol, 1.83 mL) was added gradually with stirring using a magnetic stirrer. Then, 4 mL of BF<sub>3</sub>O(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub> catalyst was added drop by drop over six hours. After copolymerization for 15 hours, indicated by the solution turning into a solid purple substance, the reaction was stopped with 1 mL of methanol. The synthesis product is then dissolved in 70 mL of chloroform. Washing was performed using a separatory funnel with distilled water until the solution's pH was neutral. The organic layer was collected and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. The remaining solvent was evaporated at room temperature and dried using a desiccator. The synthesis product became a solid and was ground using a pestle and mortar until it became powdery. The obtained PEAE copolymer was characterized by solubility testing, melting point analysis, molecular weight determination with an Ubbelohde viscometer, and FTIR analysis.

### 2.3 Synthesis of Coating Film Chitosan-Gelatin-PEAE

The film preparation procedure follows the modification of the research conducted by Jeannine et al. (2018). One gram of chitosan was dissolved in 50 mL of distilled water containing 1% glacial acetic acid (GAA) and stirred until homogeneous using a magnetic stirrer. The gelatin solution was prepared by dissolving 4 g of gelatin in 50 mL of distilled water and stirring until homogeneous using a magnetic stirrer. The two solutions, chitosan (50 mL, CH50) and gelatin (50 mL, GEL50), were mixed until homogeneous. Then, 5 mL of glycerol and PEAE copolymer with varying concentrations of 1.25%, 2.5%, and 3.75% (w/v) in DMSO were added. The film-forming solution was cast into Petri dishes and then dried in an oven at 50-60°C. The formed films were able to be peeled off and characterized, including Scanning Electron Microscopy (SEM) analysis, tensile strength (TS), elongation at break percent (E%), contact angle, and antibacterial activity.

## 2.4 Characterization

### 2.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

The synthesized PEAE and chitosan-gelatin-PEAE coating films were analyzed using an FTIR Agilent Cary 630 instrument to identify the presence of functional groups. The FTIR spectra were recorded at room temperature with a 400-4000  $\text{cm}^{-1}$  wavenumber range.

### 2.4.2 Scanning Electron Microscope (SEM)

The synthesized chitosan-gelatin-PEAE coating films were analyzed using a Hitachi SU3900 SEM instrument to examine their morphological structure. Cross-sectional micrographs of the films were obtained with magnifications ranging from 250x to 10,000x. Images were documented using an acceleration voltage of 5 kV. Four samples with different film formulations were analyzed.

### 2.4.3 Molecular Weight

The procedure for determining the molecular weight of PEAE refers to the modification of the research conducted by Habibah et al. (2013) using the viscosity method. The preparation was carried out by dissolving the PEAE copolymer in various concentrations (0.002, 0.004, 0.006; 0.008 g/mL) in 20 mL of chloroform, requiring 0.04, 0.08, 0.12, 0.16 grams of the PEAE copolymer. The flow time was measured using a stopwatch, recording the time for the solvent and the PEAE solution to flow from the upper to the lower boundary of the Ubbelohde viscometer tube. The flow time data obtained was used to determine the relative viscosity, reduced viscosity, and specific viscosity for each PEAE concentration variation. A graph is then plotted to show the relationship between concentration and reduced viscosity, yielding a linear equation where the intercept is considered the intrinsic value. The molecular weight was determined using the Mark-Houwink-Sakurada equation as follows:

$$[\eta] = K \cdot M^\alpha \quad (1)$$

Explanation:

$[\eta]$  = intrinsic viscosity

$K$  = specific constants for polymer and solvent

$M$  = polymer molecular weight (Da)

$\alpha$  = specific constants for polymer and solvent

### 2.4.4 Contact Angle

The contact angle testing procedure refers to the research conducted by Celia et al. (2013). The films that have been prepared (control and PEAE concentration variations) were cut into 1 x 1 cm sizes. The film was placed on a flat surface, and distilled water was dropped onto it using a syringe.

Documentation was taken using a 3-in-1 fish-eye camera to obtain clear images. The angle formed by the water droplet was measured using the ImageJ application, assisted by angular lines. The contact angle was calculated using Young's equation as follows:

$$\gamma_{LV} \cdot \cos\theta = \gamma_{SV} - \gamma_{SL} \quad (2)$$

With a correction factor for roughness ( $\beta$ ) and heterogeneity ( $f1$  dan  $f2$ )

$$\beta \cdot \gamma_{LV} \cdot \cos\theta = \gamma_{SV} - \gamma_{SL} \quad (3)$$

$$\gamma_{LV} \cdot \cos\theta = f1 (\gamma_{SV} - \gamma_{SL}) + f2 (\gamma_{SV} - \gamma_{SL}) \quad (4)$$

Explanation:

$\gamma_{LV}$  = surface tension (air-liquid interface)

$\theta$  = contact angle

$\gamma_{SV}$  = interfacial tension (solid-air)  
(surface free energy of the solid)

$\gamma_{SL}$  = interfacial tension (solid-liquid)

### 2.4.5 Antibacterial Activity

The preparation of bacterial culture stock on solid media involved dissolving 2 g of nutrient agar (NA) in 100 mL of distilled water until homogeneous. The media and other equipment were sterilized using an autoclave for 45 minutes and exposed to UV light. The media was poured into test tubes at a 30° angle until it solidifies. The test bacterial colonies (*Staphylococcus aureus* and *Escherichia coli*) were inoculated onto the slanted surface of the medium in a sterile laminar flow cabinet using an inoculating needle in a zigzag pattern. The test tubes were incubated in an incubator at 37 °C for 18-24 hours.

Preparing bacterial culture stock in liquid media involved dissolving 1.4 g of nutrient broth (NB) in 100 mL of distilled water until homogeneous. The media and other equipment were sterilized using an autoclave for 45 minutes and exposed to UV light in a laminar flow cabinet. Bacteria from the solid media stock were taken with one loop of each and transferred into an Erlenmeyer flask containing 10 mL of sterile NB media. The flask was then incubated using an incubator shaker for 6 hours until the bacterial media became turbid, indicating bacterial growth.

Preparing the antibacterial activity test solution involved dissolving 0.7 g of NB in an Erlenmeyer flask with 50 mL of distilled water until homogeneous. The media and other equipment were sterilized using an autoclave for 45 minutes and exposed to UV light in a laminar flow cabinet. The bacterial suspension was prepared by adding 1% of the liquid bacterial stock to an Erlenmeyer flask containing sterile NB media. The flask was then incubated using an incubator shaker until the bacterial turbidity matched the 0.5 McFarland standard. Standardization was done by measuring the bacterial suspension until an absorbance value of 0.08-0.1 was achieved at a wavelength of 600-625 nm. Three

erlenmeyer flasks were prepared for each bacterial suspension of *Staphylococcus aureus* and *Escherichia coli*: one as a negative control (containing bacterial suspension), one as a positive control (bacterial suspension plus chloramphenicol antibiotic), and one for the sample test (bacterial suspension plus coating film sample). Nine coating films with various PEAE concentrations, sized 1x1 cm, were placed into the bacterial suspension. They were then incubated using an incubator shaker, and absorbance was measured after a 12-hour contact time using a UV-Vis spectrophotometer at a wavelength of 600 nm.

The antibacterial activity of the coating film samples using the turbidimetric method was analyzed by calculating the percentage of bacterial growth inhibition using the formula:

$$\% \text{ inhibition} = \frac{OD \text{ control negative} - OD \text{ sample}}{OD \text{ control negative}} \times 100\% \quad (5)$$

Explanation:

OD control negative: the absorbance value of control negative

OD sample : the absorbance value of the sample testing medium

### 3. RESULT AND DISCUSSION

#### 3.1 Copolymer Eugenol-Allyl Eugenol (PEAE)

The synthesis procedure of eugenol/allyl eugenol (PEAE) copolymer refers to modifying the research previously conducted by Ngadiwiyana et al (2022). The synthesis of the eugenol/ allyl eugenol (PEAE) copolymer involves a cationic addition polymerization reaction between eugenol and the cross-linking agent allyl eugenol. A concentration of 10% allyl eugenol relative to the molar amount of eugenol is chosen because it represents the optimal concentration variation that results in a denser and stronger copolymer with the lowest swelling degree.

The BF<sub>3</sub> catalyst accelerates the copolymerization reaction, purging the system under inert conditions using nitrogen gas (N<sub>2</sub>). The purpose of purging with N<sub>2</sub> is to remove oxygen that could interfere with and slow down the polymer formation during the polymerization reaction.

Cationic addition polymerization occurs in three stages: initiation, propagation, and termination, as shown in **Figure 1**. The BF<sub>3</sub> catalyst is added as an acid initiator in the initial initiation stage. It reacts with the eugenol or allyl eugenol monomer to form a carbocation or carbonium ion, marked by the addition to the vinyl group (-CH=CH<sub>2</sub>-). This carbocation is a secondary intermediate carbocation, more stable than a primary one. The formation of a reddish-brown solution indicates this process. The second stage is propagation, where the copolymerization process occurs repeatedly. The vinyl group on the eugenol or allyl eugenol monomer bonds with the carbocation through electron pairs, producing a new carbocation. The final stage is termination, where the copolymerization reaction is stopped by adding methanol, which acts as a nucleophile. The carbocation becomes inactive due to the electron pair donation from the oxygen atom (electronegative) in methanol.

The FTIR spectrum analysis of the eugenol-allyl eugenol (PEAE) copolymer shown in **Figure 2** indicates a change due to the disappearance of the vinyl group (-CH=CH<sub>2</sub>-) as the C=C alkene bond in the wavelength range of 1678-1640 cm<sup>-1</sup>. This change is caused by the addition polymerization reaction initiated by the acid catalyst (BF<sub>3</sub>O(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>) on the vinyl groups within the eugenol and allyl eugenol structures. **Table 1** compares the FTIR absorption analysis for eugenol, allyl eugenol, and the eugenol-allyl eugenol (PEAE) copolymer.

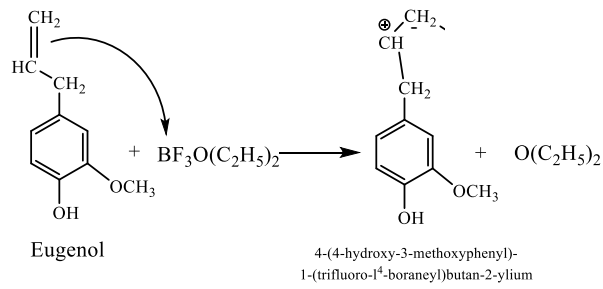
**Table 1.** Absorption regions of functional groups in the FTIR spectra of eugenol, allyl eugenol, and PEAE

Functional Groups	Wavenumber (cm <sup>-1</sup> )		
	Eugenol	Allyl Eugenol	PEAE
Stretching O-H	3518.61	-	3503.7
Stretching C-H sp <sup>3</sup>	2840.23-2974.41	2832.77-2974.41	2840.23-2959.5
Stretching C=C alkene	1640.02	1640	-
Stretching C-O-C	1228.6	1221.03	1205.05
Stretching C-O phenol	1118.2	-	1118.2
Stretching C-H sp <sup>2</sup> aromatic	745.46	745.5	752.9

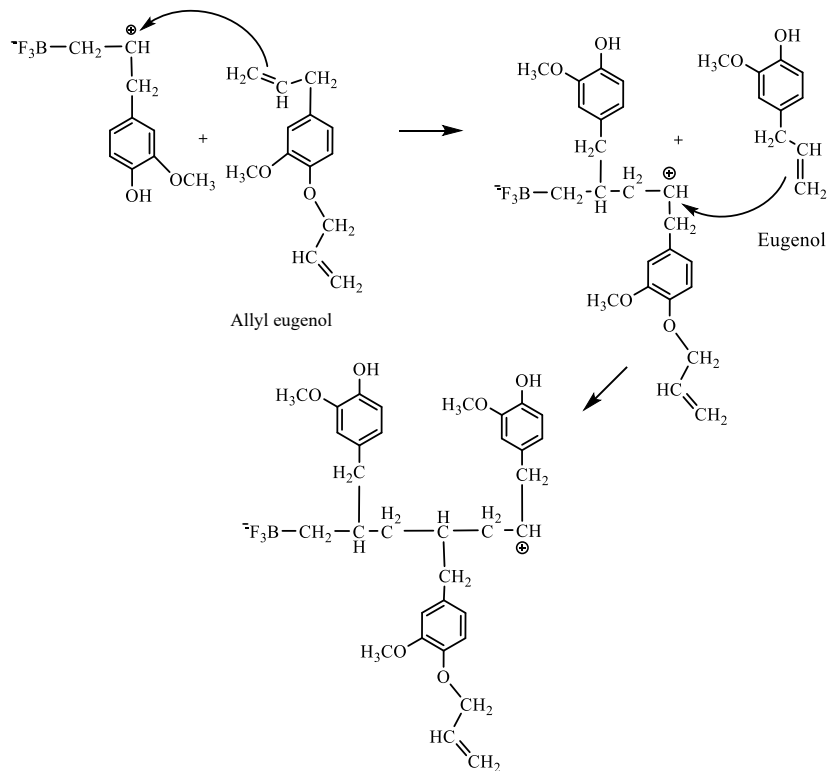
The brown solid of eugenol-allyl eugenol (PEAE) copolymer was successfully synthesized with a yield of 94.9061%. The physical properties of PEAE, including melting point, solubility, and molecular weight, were analyzed. PEAE has a melting point of 95-98°C and a molecular weight of 9,553.979 Da. PEAE is soluble in solvents such as methanol, ethanol,

toluene, dichloromethane, DMSO, ethyl acetate, and chloroform, but it is insoluble in aqueous solvents and n-hexane. Solubility is based on the principle of like dissolves like; polar compounds dissolve in polar solvents, and nonpolar compounds dissolve in nonpolar solvents.

Initiation stage



Propagation stage



Termination stage

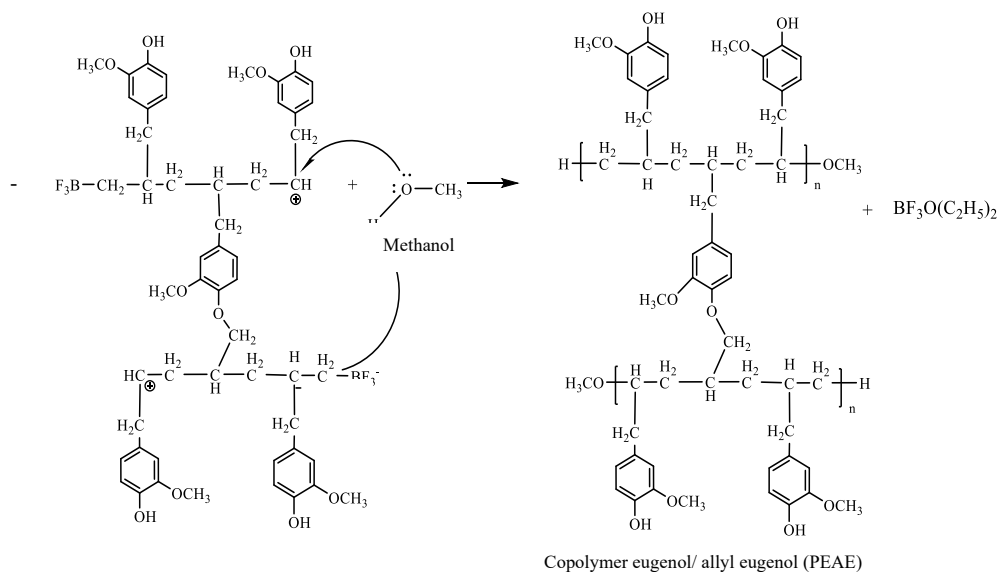
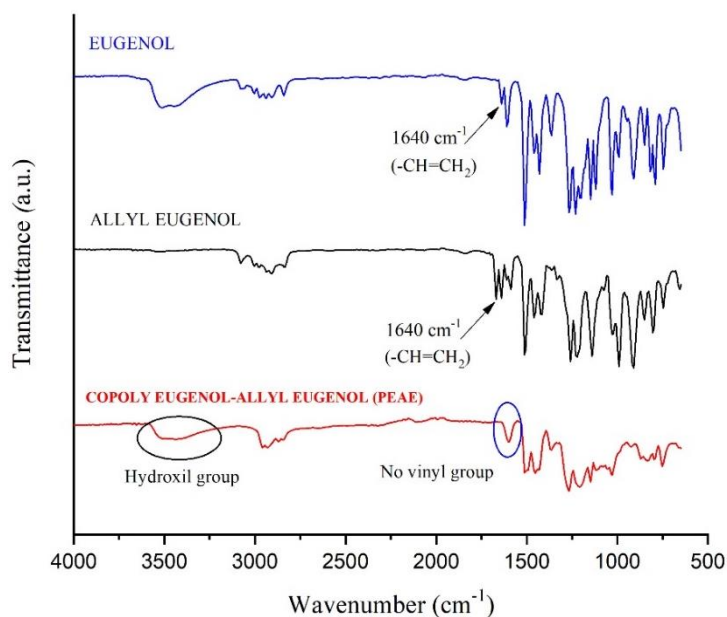


Figure 1. Copolymerization reaction in the synthesis of PEAE

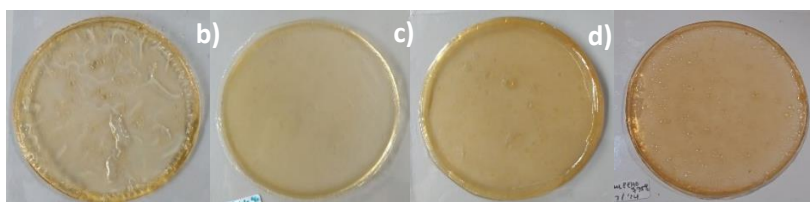


**Figure 2.** FTIR spectra of eugenol, allyl eugenol, and PEAE

### 3.2 Coating Film of Chitosan-Gelatin-PEAE

The coating film preparation procedure refers to modifications based on research by Bonilla et al. (2018). Four variations of coating films were made with sample codes: control, PEAE 1.25, PEAE 2.5, and PEAE 3.75. The control film composition is a

chitosan, gelatin, and glycerol mixture without adding the antibacterial active compound eugenol-allyl eugenol (PEAE) copolymer. The compositions for the PEAE 1.25, 2.5, and 3.75 films consisted of the control film base mixture with varying concentrations of PEAE at 1.25%, 2.5%, and 3.75% in 10 mL of DMSO. The resulting coating films were thin yellowish-brown sheets, as shown in **Figure 3**.



**Figure 3.** Coating film of control (a); PEAE 1.25 (b); PEAE 2.5 (c); PEAE 3.75 (d)

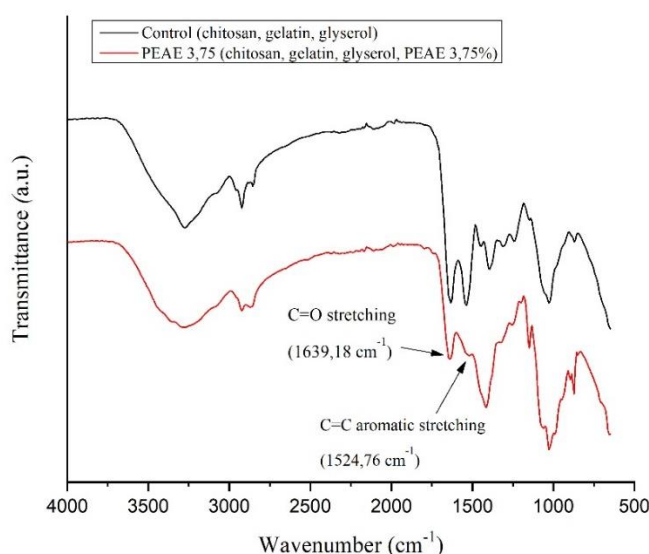
Interactions between the composite film matrix can be observed from the IR spectral analysis results shown in Figure 4, comparing the IR spectra of the control film with the PEAE 3.75 film. In the control coating film, secondary interactions (electrostatic interactions and hydrogen bonding) occur between chitosan, gelatin, and glycerol. The negatively charged carboxyl groups of gelatin interact with the positively charged amino groups ( $\text{NH}_3^+$ ) of chitosan in acidic conditions. Other functional groups such as  $-\text{OH}$ ,  $-\text{NH}_2$ , and  $-\text{COOH}$  from gelatin also interact with  $-\text{OH}$  and  $-\text{NH}_2$  groups on the chitosan chain. The wavenumber  $1027.52 \text{ cm}^{-1}$  indicated secondary interactions between glycerol, chitosan and gelatin. The hydroxyl groups in glycerol form

hydrogen bonds with the carbonyl and amino groups of chitosan and gelatin<sup>21</sup>.

The success of the PEAE 3.75 coating film synthesis was evidenced by the appearance of peaks at a wavenumber of  $1639.18 \text{ cm}^{-1}$ , identified as the absorption band for  $\text{C}=\text{O}$  stretching, and at  $1524.76 \text{ cm}^{-1}$ , corresponding to aromatic  $\text{C}=\text{C}$  groups<sup>22</sup>. Additionally, there is a decrease in the intensity of the  $\text{C}=\text{O}$  stretching,  $\text{N}-\text{H}$  bending,  $\text{C}-\text{O}$  stretching, and  $\text{C}-\text{N}$  stretching bands due to interactions between PEAE and other components through electrostatic interactions<sup>23</sup>. **Table 2** presents the absorption data of functional groups for the control film and PEAE 3.75 film using FTIR.

**Table 2.** Absorption regions of functional groups in the FTIR spectra of the control film and PEAE 3.75 film

Functional Groups	Wavenumber (cm <sup>-1</sup> )	
	Control	PEAE 3,75
Stretching -OH dan stretching -NH	3273.08	3289.06
Stretching C-H alkane	2923.08; 2854.09	2922.24; 2868.40
Stretching C=O	1634.98	1639.18
Bending N-H	1534.86	1524.76
Stretching C=C aromatic	-	1524.76
Stretching C-O, C-N	1242.91	1256.37
Bending C-H	1449	1417.07
Bending O-H	1394.35	1417.07
Stretching C-O-C	871.87; 1027.52;	871.88; 1029.21;
	1147.84	1148.68
Stretching -OH	1027.52	1029.21



**Figure 4.** FTIR spectra of control and PEAE 3.75 film

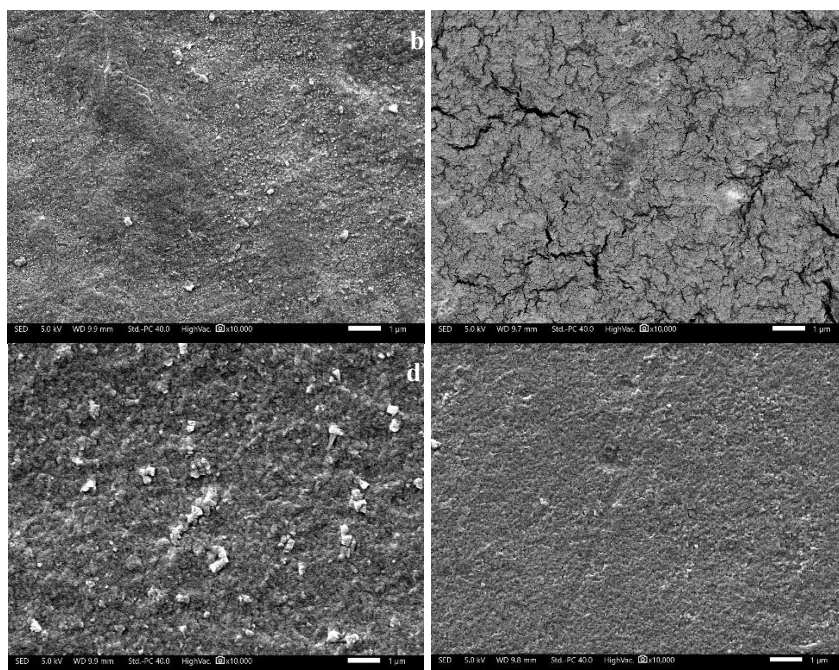
The film obtained was a thin, transparent sheet with a yellowish-brown color; the color darkens with increasing PEAE concentration. Based on the SEM characterization results shown in Figure 5, there was a difference in morphological structure between the control film and films with varying concentrations of PEAE. Films with PEAE variations exhibited a porous structure due to the hydrophobic nature of the PEAE hydrocarbon chains, which reduces polymer chain interactions, resulting in a more open film structure.

The results of tensile strength (TS) and elongation percent (E%) tests for the control film, PEAE 1.25, PEAE 2.5, and PEAE 3.75, are presented in **Table 3**. The decrease in TS and increase in E% values are due to PEAE acting as a plasticizer, which reduces intermolecular forces along the polymer chains, thus enhancing flexibility. The contact angle analysis, shown in Figure 6, indicates that all four samples are hydrophilic, with contact angles less than 90°.

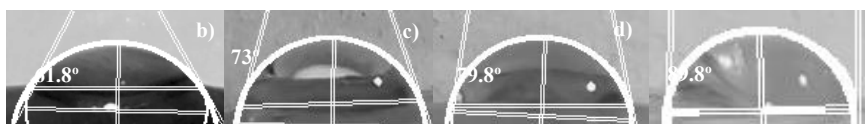
**Table 3.** Tensile strength (TS) and elongation percent (E%) data for coating film

Sample Code	Composition	Tensile Strength (Mpa)	Elongation Percent (%)
Control	CH50:GEL50	30.4	4.3
PEAE 1.25	CH50:GEL50:PEAE 1.25%	24.81	5.7
PEAE 2.5	CH50:GEL50:PEAE 2.5%	15.48	14.8
PEAE 3.75	CH50:GEL50:PEAE 3.75%	15.04	3.2





**Figure 5.** SEM test of the morphological structure of the control coating film (a), PEAE 1.25 (b), PEAE 2.5 (c), PEAE 3.75 (d) at 10,000x magnification



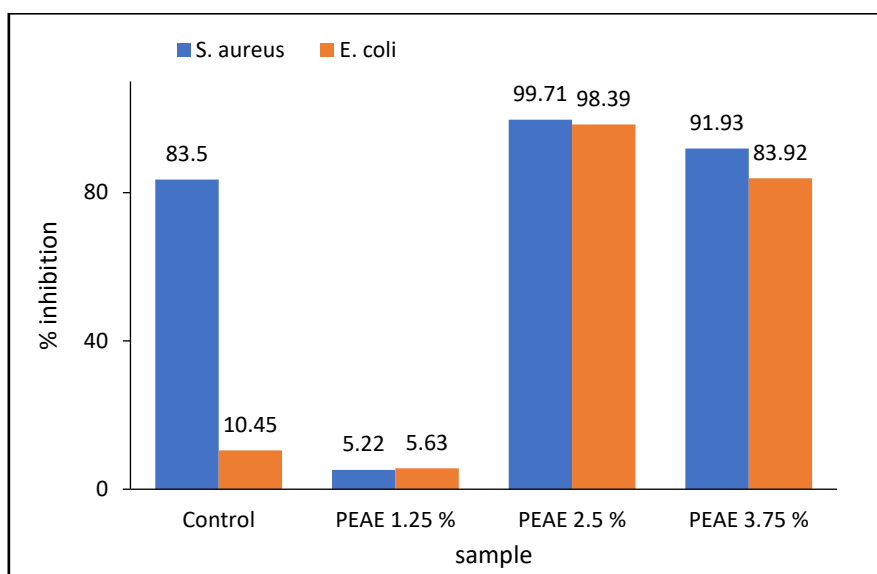
**Figure 6.** Contact angle of the control coating film (a), PEAE 1.25 (b), PEAE 2.5 (c), and PEAE 3.75 (d)

### 3.3 Antibacterial Activity of Coating Film Chitosan-Gelatin-PEAE

The antibacterial activity test procedure using the turbidimetric method refers to modifications of research conducted by Cahyaningsih et al. (2015) and Frengky et al. (2019). The bacteria used in the analysis were *Staphylococcus aureus* (a gram-positive bacterium) and *Escherichia coli* (a gram-negative bacterium). This choice is because the film matrix components, namely chitosan and eugenol, are known to inhibit the growth of pathogenic bacteria, including both gram-positive and gram-negative bacteria. The analysis was conducted by preparing test media for each bacterium using three Erlenmeyer flasks: one as a negative control, one as a positive control, and one for the sample media. The negative control contains a bacterial suspension that meets the 0.5 McFarland standard with an absorbance range of 0.08–0.1. The

positive control contains a bacterial suspension supplemented with chloramphenicol, a broad-spectrum antibiotic effective against gram-positive and gram-negative bacteria<sup>24</sup>. The sample media contains bacterial suspension supplemented with coating film samples of various PEAE concentrations, sized 1x1 cm, a total of 9 pieces to maximize the contact surface area with the bacterial suspension. The test samples were incubated using an incubator shaker to optimize bacterial growth by evenly distributing nutrients in the liquid media and ensuring oxygen availability, maintaining aerobic conditions<sup>25</sup>. Although *Staphylococcus aureus* and *Escherichia coli* are facultative anaerobes, they can grow well in aerobic conditions.<sup>26</sup> The data presented in Figure 7 shows a diagram of the percentage of inhibition of *Staphylococcus aureus* and *Escherichia coli* within 12 hours for the sample codes: control, PEAE 1.25; PEAE 2.5; and PEAE 3.75.





**Figure 7.** Diagram of the percentage of inhibition of *Staphylococcus aureus* and *Escherichia coli*

Based on the results, the best inhibition percentage was achieved with the PEAE 2.5 sample, showing 99.71% inhibition of *Staphylococcus aureus* and 98.39% inhibition of *Escherichia coli*. Overall, the results indicated that the samples were more effective at inhibiting *Staphylococcus aureus* growth than *Escherichia coli*. The matrix responsible for providing the antibacterial effect in the coating film is the copolymer eugenol-allyl eugenol (PEAE) and chitosan. Chitosan contains amino polysaccharides and lysozyme enzymes that play a role in inhibiting bacterial growth<sup>27</sup>. The electrostatic interaction between chitosan and bacteria involves a bond between the positively charged protonated amino group in chitosan ( $-\text{NH}_3^+$ ) and the negatively charged carboxylate group ( $-\text{COO}^-$ ) on the bacterial cell surface. Free hydroxyl groups in the copolymer eugenol-allyl eugenol (PEAE) play a crucial role in providing the antibacterial effect. The inhibition process involving electrostatic interaction occurs when the free hydroxyl groups bind to the negative charge on the bacterial cell surface, and cellular proteins, altering membrane fatty acids, and inhibiting enzyme activity.

#### 4. CONCLUSIONS

The synthesis of the eugenol-allyl eugenol copolymer (PEAE) was successfully achieved, resulting in a brown solid with a yield of 94.9061%, a molecular weight of 9,553.979 Da, and a melting point range of 95–98°C. PEAE was soluble in solvents such as methanol, ethanol, toluene, dichloromethane, DMSO, ethyl acetate, and chloroform but insoluble in distilled water and n-hexane. The synthesis of chitosan-gelatin coating films with varying concentrations of the eugenol-allyl eugenol copolymer (PEAE) was completed, with sample codes including

control film, PEAE 1.25, PEAE 2.5, and PEAE 3.75, all producing explicit thin films with a yellow-brown hue. As more PEAE concentration was added, the coating films exhibited more porous morphology, increased hydrophobicity, decreased tensile strength (TS), and increased elongation percentage (E%)—films with the greatest hydrophobicity to add PEAE 3.75 % with a contact angle of 89,8 °. The best tensile strength in adding 1.25% PEAE was 24.81 MPa, while the largest (5E) papers were in the film with a 2.5% PEAE with % E was 14.8%. The antibacterial activity of chitosan-gelatin-PEAE-based coating films was successfully tested using the turbidimetric method. The best antibacterial activity was observed with the PEAE 2.5 film containing 2.5% PEAE concentration, with inhibition percentages against *Staphylococcus aureus* and *Escherichia coli* of 99.71% and 98.39%, respectively.

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