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Synthesis of copolymer eugenol crosslinked with divinyl benzene and preliminary study on its antibacterial activity

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Abstract. Copolymer eugenol-divinyl benzene (PEDVB) was successfully synthesized via eugenol copolymerization with divinyl benzene using BF_3 as a catalyst. The resulting copolymer was characterized using FTIR. Its antibacterial activity was tested using agar disc diffusion method by calculating the inhibitory zone diameters against *Escherichia coli* as a gram-negative bacteria and *Staphylococcus aureus* as a gram-positive bacterium. In addition, ampicillin and DMSO were used as a positive control and a negative control, respectively. The results showed that PEDVB performs antibacterial activity with slow response category.
Keywords: eugenol, divinylbenzene, copolymer, antibacterial

1. Introduction

Eugenol (4-allyl-2-methoxyphenol) is a natural compound and the main component in clove oil (70-90%) [1]. Eugenol is also found in various essential oils, such as cinnamon and nutmeg oil [2]. Eugenol compounds can be easily isolated by adding bases to produce eugenolate compounds which can dissolve in water. High purity Eugenol can be recovered by adding an acid.

Eugenol is reported to have antibacterial activity with a “moderate-strong inhibitory" category in inhibiting the growth of several test microorganisms, such as *Escherichia coli*, *Aeromonas hydrophila* dan *Salmonella typhi* [3-6]. Antibacterial activity arises due to the interaction between eugenol and bacterial cell membrane [3, 7]. Although it has anti-bacterial activity, eugenol has a liquid form that limits its use in various applications, such as in hygienic applications, textiles, food packaging and water purification systems.

Based on its chemical point of view, eugenol has three functional groups, namely hydroxyl, methoxy and allyl groups so that eugenol can be modified into various derivative compounds. Through the modification of the allyl group, eugenol can be polymerized in the presence of an acid catalyst, such as concentrated sulphuric acid or BF_3 to form a solid homopolymer (polyeugenol) or even a solid copolymer if eugenol is crosslinked with other compounds which also have an allyl group [8, 9]. Divinyl benzene (DVB) is one compound that has two allyl terminals which can be used in copolymerization. This copolymerization produces eugenol-DVB copolymer (PEDVB) that have a net-like structure [10]. Net-like polymer structure is important because it make the polymers become mechanically strong and resistant to heat and solvents attack.

Having a net-like structure, PEDVB is interesting to study for its properties, such as solubilities and its antibacterial activity for further applications. Therefore, the aim of this work was to focus on the synthesis of PEDVB copolymer in the presence of BF_3 catalyst and the preliminary study on its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

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2. Experimental

2.1. Reagents

Eugenol (for synthesis), boron trifluoride-diethyl ether complex (for synthesis), divinyl benzene (for synthesis), methanol (for analysis), chloroform (for analysis), natrium sulphate anhydrite, dimethyl sulfoxide (for analysis), nutrient agar (for microbiology GranuCult™), pepton, yeast extract and ampicillin were purchased from Merck. Cultures of Staphylococcus aureus (S. aureus) (ATCC 25923) and Escherichia coli (E. coli) (ATCC 25922) were obtained from Biochemistry Laboratory of Diponegoro University Semarang. All compounds were used as received without further purification.

2.2. Synthesis of copolymer PEDVB

Eugenol (5.8 g, 35 mmol) and DVB (0.87 g, 6.6 mmol) were added to chloroform solution in the three-neck flask. The mixture was then stirred while adding BF_3-diethyl ether complex (1 mL) dropwise under N_2 atmosphere. The mixture remains stirred overnight and quenched with methanol. The mixture formed was dissolved in diethyl ether and washed using distilled water until it reached neutral pH. The organic layer was separated and dried using anhydrous Na_2SO_4. The solvent was evaporated with a rotary evaporator and the residue was dried in a desiccator. The formed polymer was weighed to find the yield. Solubility of copolymer products was characterized in various types of solvents. Number and weight of average molecular weights were determined using Ubbelohde viscometer. FTIR spectra were recorded using Frontier-FTIR spectrophotometer.

2.3. Bacterial cultures

E. coli and *S. aureus* bacteria were grown in agar media. The procedure was started with mixing yeast extract (0.05 g), peptone (0.25 g) and nutrient agar (1.5 g) into distillate water (100 mL). The homogeneous mixture was sterilized using an autoclave for 45 minutes along with the test tube and ose needle. A total of 5 mL of media was poured into 3 test tubes and allowed to condense at room temperature with the position of the test tube tilted 30°C to the flat plane. After compacting, the test bacteria to be inoculated on the sloping agar media were taken using a sterile ose needle. The bacterial colonies that have been taken were then inoculated by scraping into the solid media. The process of bacterial inoculation on oblique media was carried out in Laminar Air Flow. Incubation of bacterial inoculation on oblique media was carried out at 37°C for 18-24 hours. The same treatment was carried out on *E. coli* and *S. aureus*.

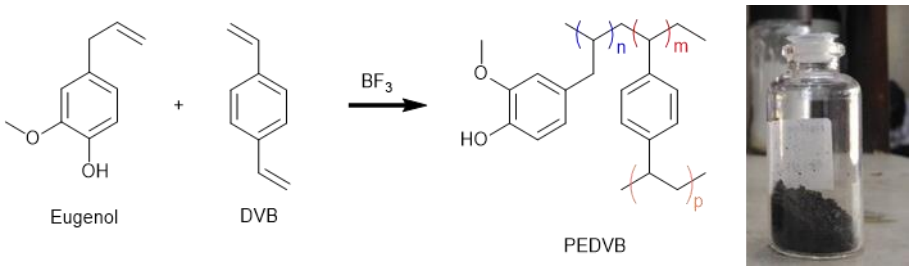
2.4. Antibacterial activity testing

A total of 10 µL test solution, namely copolymer PEDVB, eugenol, DVB, DMSO solution as a negative control, Ampicillin solution as a positive control were dripped each on a disc paper and allowed to stand for 1 minute until the test solution diffused perfectly. The disc paper containing the test solution was placed on the surface of the test media. The test medium was incubated at 37°C for 24 hours. Observation of antibacterial was activity carried out at 12 and 24 hours' incubation time by measuring the clear zone formed around the disc paper. The clear area was an indication of the sensitivity of bacteria to antibiotics or other antibacterial materials used as test material expressed in the inhibitory zone diameter. The diameter of the inhibitory zone was measured using a calliper run in units of millimetres by means of the overall diameter minus the diameter of the disc paper.

3. Results and Discussion

The chemical structure of eugenol has been tried to be modified to improve its properties. The solubility of monomer eugenol in various solvents makes this compound has disadvantages, for example as adsorbents in water treatment. Thus, in this study copolymerization with DVB was carried out to produce molecules with a net-like structure to improve their physical properties. The copolymerization reaction between eugenol and DVB using chloroform as solvent in the presence of BF_3 catalyst was used for the synthesis of PEDVB copolymers to obtain a dark solid with a yield of 90%. Synthetic routes

are shown in scheme 1. The resulting PEDVB is not soluble in water, methanol, ethanol; has low solubility in DMSO but completely soluble in chloroform. Having a solid form and not soluble in several solvents, PEDVB has better potential to be used in various applications, such as in hygienic applications, textiles, food packaging, and water purification systems, compared to eugenol. Molecular weight of PEDVB was characterized using Ubbelohde and was found to be 37,613 g mol^{-1}.



Scheme **1.** Synthetic reaction of PEDVB and dark solid appearance of PEDVB.

FTIR spectra of synthesized PEDVB compared to eugenol and DVB reactants is shown in Fig. 1. It is clear that the absorption of vinyl groups is (-C=C-) in the area of 1680-1620 cm^{-1} area does not appear because they are used bind to form polymers. This shows that the PEDVB copolymer has been formed.

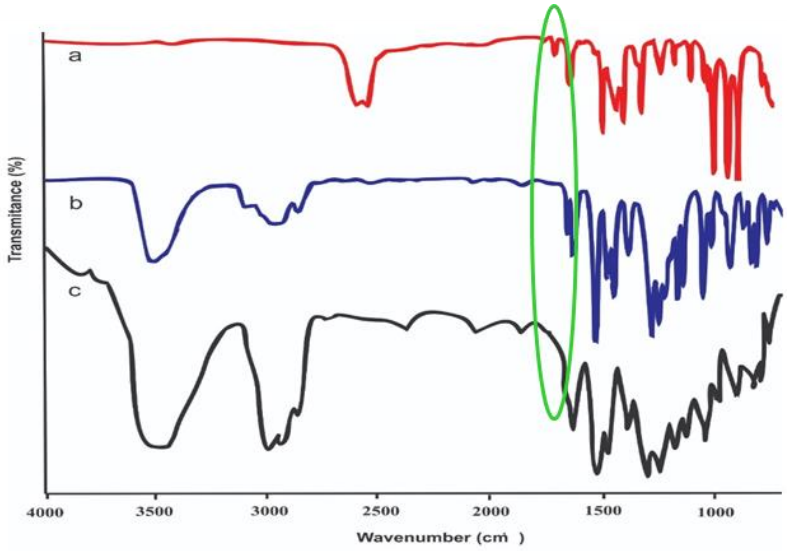


Figure 1. Comparison of FTIR spectra for (a) DVB (red line), (b) pure eugenol (blue line) and (c) PEDVB sample (black line).

The antibacterial ability of PEDVB was tested using agar disc diffusion method and compared to the initial monomers (eugenol and DVB) in vitro against pathogenic gram-positive bacteria *S. aureus* and gram-negative *E. coli*. Antibacterial activity was analysed by measuring the diameter of the clear zone against the test bacteria. As a positive control, ampicillin was used because of its ability to inhibit the growth of both bacteria through inhibition of wall formation and cell membrane permeability [11]. Meanwhile, as a negative control DMSO was used because of its ability to dissolve test sample compounds and is a solvent that has no antibacterial properties. Antibacterial activity test from each sample was carried out in a concentration of 20 mg mL^{-1}, while positive control was carried out in a concentration of 0.5 mg mL^{-1}. The compounds that have been inoculated into the test media were then observed at 12 and 24 hours of incubation time to determine the effective time of PEDVB ability to inhibit bacteria growth.

Table 1. Antibacterial activity test results of PEDVB and comparative compounds.

No.	Compound	Inhibitory zone diameter (mm)			
		E. coli		*S. aureus*	
		12 hours	24 hours	12 hours	24 hours
1	PEDVB	0.87	0.52	2.27	1.17
2	Eugenol	1.67	1.40	2.62	1.63
3	DVB	0	0	0	0
4	Ampicillin (+)	21.82	21.88	6.1	3.62
5	DMSO (-)	0	0	0	0

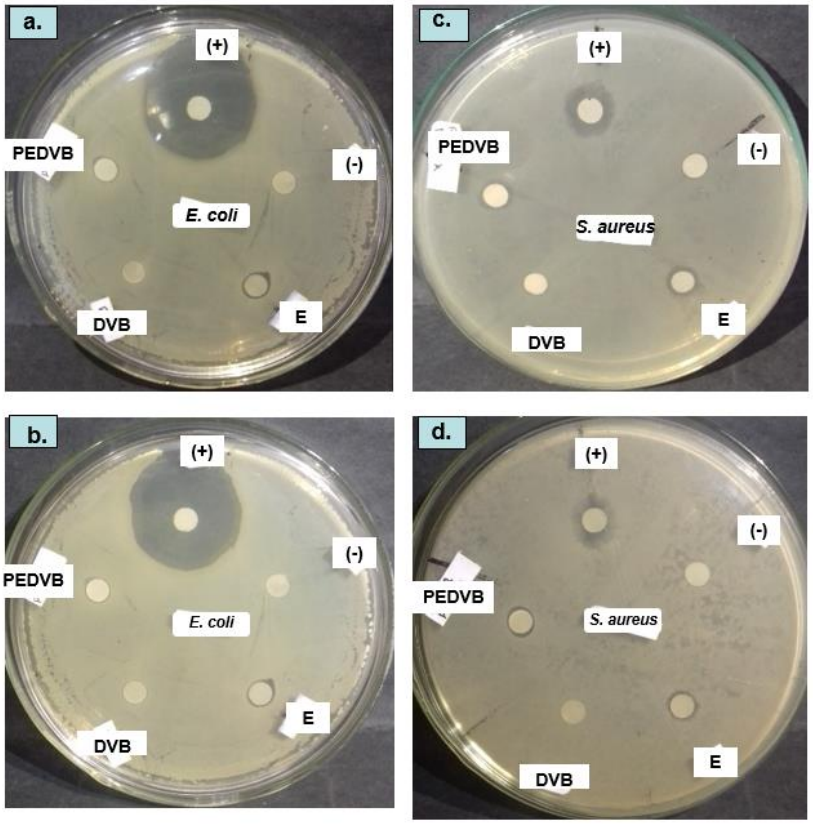


Figure 2. Results of agar disc diffusion test of copolymer PEDVB, eugenol, DVB, ampicillin (+) and DMSO (-) against (a) E. coli after 12 h; (b) E. coli after 24 h; (c) S. aureus after 12 h and (d) S. aureus after 24 h of incubation at 37°C.

The results of the PEDVB antibacterial activity test and comparative compounds are shown in table 1 and Fig. 2. In contrast to eugenol, the DVB compound as one of the copolymer composing monomers did not show antibacterial activity. PEDVB and eugenol copolymers have the greatest inhibition at 12 hours contact time, while at 24 hours contact time there is a decrease in inhibitory ability which shows PEDVB and eugenol compounds have an inhibitory time span of no more than 12 hours. The sensitivity of PEDVB is greater in gram-positive bacteria (*S. aureus*) than that of in gram-negative bacteria (*E. coli*), as evidenced by the greater inhibition zone value in *S. aureus* compared to *E. coli*. This difference

in sensitivity is probably caused by outer membrane factor. Gram negative bacteria have outer membrane so that foreign molecules do not easily diffuse through the cell wall. As a result, gram negative bacteria are more resistant to antibacterial material than gram positive bacteria. Data also showed that PEDVB (20 mg L^{-1}) has lower activity compared to eugenol monomer at similar concentration. Since polymers have a much higher molecular weight, they cannot spread easily to bacterial cell walls compared to their monomers. According to Pan *et al.* [12], with a inhibition zone value of <3 mm in both bacteria, PEDVB is categorized as an antibacterial with a slow response.

4. Conclusion

PEDVB copolymer was successfully synthesized using eugenol and DVB monomers in the presence of BF_3 which produced dark solid with 90% yield. The resulting PEDVB is not soluble in water, methanol, ethanol; has low solubility in DMSO but completely soluble in chloroform. Molecular weight of PEDVB using Ubbelohde is 37,613 g/mol. PEDVB has antibacterial activity against *E. coli* and *S. aureus* bacteria with a slow response category.

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