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Synthesis and study of antibacterial activity of polyeugenol

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Abstract. Research on synthesis and study of antibacterial activity of polyeugenol has been carried out. The synthesis of polyeugenol was carried out by cationic polymerization using a BF_3 catalyst at room temperature under nitrogen atmosphere. The resulting polyeugenol is brownish and not soluble in water but in acetone, chloroform, DMSO and benzene solvents. Molecular weight of Polyeugenol using Ubbelohde is 9728 g mol^{-1} to find a degree of polymerisation (DP) of 60. Antibacterial test was carried out by comparing the activity of polyeugenol synthesized against *Escherichia coli* and *Staphylococcus aureus* with disc diffusion method. In this study, ampicillin was used as a positive control and DMSO was used as a negative control. The test result showed that polyeugenol still has antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria with a slow response category.

Keywords: eugenol, polymer, polyeugenol, antibacterial

1. Introduction

The development of the polymer industry provides many opportunities for the creation of new materials that are useful in various fields such as in water treatment, health, and other industries [1-3]. Most polymer materials used do not have antibacterial properties that require further modification to obtain antibacterial properties. This results in increasing steps and costs in order to get the antibacterial polymer material.

Eugenol is a natural material that can be obtained from various natural ingredients, such as clove oil, nutmeg oil, cinnamon bark and many other plants [4]. In clove oil, eugenol is the main component with content reaching 70-90% [5]. Furthermore, eugenol is easily separated from other components by adding bases. Eugenol is reported to have antibacterial activity that works through interactions with bacterial cell membranes [6]. The antibacterial activity of eugenol is included in the level of "moderate-strong inhibitory" where eugenol inhibits the growth of test microorganisms, such as *Aeromonas hydrophila*, *Enterococcus faecalis* and *Salmonella typhi* [6-9].

Based on its chemical structure, eugenol has benzene ring, allyl, methoxy and hydroxyl functional groups to make it possible to obtain other more useful eugenol derivatives. Allyl group in eugenol can act as a site for cationic polymerization reactions producing eugenol polymers (polyeugenol). Eugenol can be polymerized cationically by employing an acid catalyst, such as concentrated sulphuric or BF_3 in a good performance [10-12].

As far as the authors understanding, the analysis of polyeugenol antibacterial properties has not been widely reported. In this present paper, we demonstrated the synthesis of polyeugenol using BF_3 catalyst and we report the antibacterial properties of polyeugenol synthesized against *Escherichia coli* and *Staphylococcus aureus*.



2. Experimental

2.1. Materials and Reagents

Eugenol (for synthesis), boron trifluoride-diethyl ether complex (BF_3 , for synthesis) methanol (for analysis), chloroform (for analysis), Sodium sulphate anhydrous, dimethyl sulfoxide (DMSO, 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). All compounds were used as received.

2.2. Synthesis of polyeugenol

Polyeugenol was obtained by adding chloroform solution to a three-neck flask then mixed with eugenol (5.8 g, 35 mmol). During the polymerization process, the system was fed with nitrogen gas at room temperature while added BF_3 in diethyl ether (1 mL) dropwise. Polymerization was carried out overnight and quenched with adding methanol (1 mL). The polymerization result was dissolved in diethyl ether and then washed using distilled water until it reached neutral pH. The organic layer was then dried with the addition of 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. Polymer product was characterized based on their solubility in various solvents, molecular weight using Ubbelohde and functional group analysis using Infrared Spectrophotometer (Frontier FT-IR).

2.3. Bacterial cultures

Preparation of stock of *E. coli* and *S. aureus* bacteria was started by making the media tilted. A total of 0.05 g of yeast extract, 0.25 g of peptone and 1.5 g of nutrients was dissolved into 100 mL of distilled water. The mixture was homogenized by stirring then 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 were inoculated on the sloping agar media 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. Minimum inhibitory testing

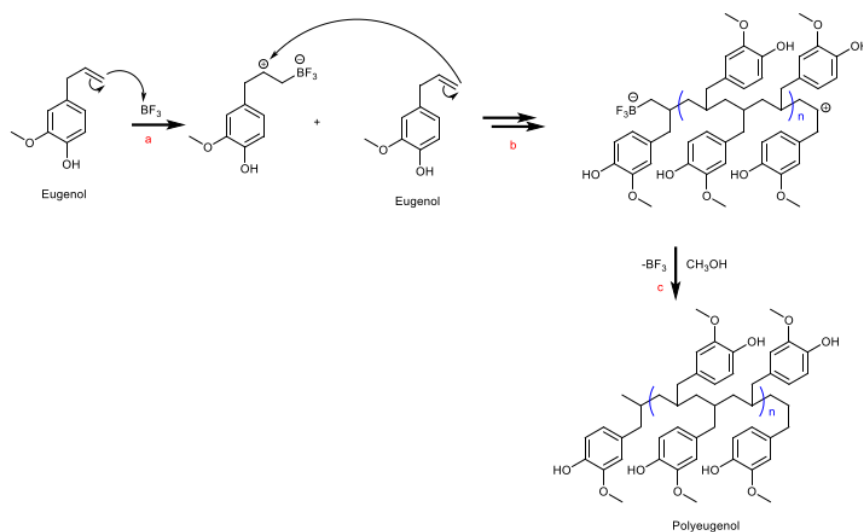
A total of 10 μL of the test solutions, which were eugenol, polyeugenol, DMSO as a negative control and ampicillin solution as a positive control, were dripped each on a disc paper and allowed to stand for 1 minute until the test solution diffused perfectly. The disc paper containing the test solution was placed on the surface of the test media. The test medium was incubated at 37°C for 24 hours. Observations of antibacterial activity were carried out at 12 and 24 hours' incubation time by measuring the clear zone formed around the disc paper. The clear zone is 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 unit of millimetres by means of measuring overall diameter minus the diameter of the disc paper.

3. Results and Discussion

3.1. Synthesis of polyeugenol

Eugenol polymerization occurs through a cationic addition polymerization process (Scheme 1) because the allyl group of eugenol undergoes an addition reaction. At the initiation stage an addition reaction occurs causing the breaking of the double bond on the allyl group in eugenol in the presence of BF_3 lewic acid catalyst and producing carbocation. At the propagation stage there is the formation of a long

polymer chain. While the termination stage occurs the termination of the growth of the polymer chain through the addition of methanol.



Scheme 1. Mechanism of eugenol polymerisation involving (a) initiation, (b) propagation and (c) termination stages.

The resulting polyeugenol is an orange solid and not soluble in water but in acetone, chloroform, DMSO and benzene solvents. Molecular weight of Polyeugenol using Ubbelohde is 9728 g mol^{-1} to find a degree of polymerisation (DP) of 60. FTIR spectra of pure eugenol and polyeugenol synthesized results are shown in Fig. 1. Polyeugenol is successfully synthesized with evidence of loss of allyl group absorption ($\text{C}=\text{C}$) at wave numbers 1637.48 cm^{-1} and reduced intensity of vinyl groups on eugenols at wavenumbers of 914.63 and 1123.05 cm^{-1} .

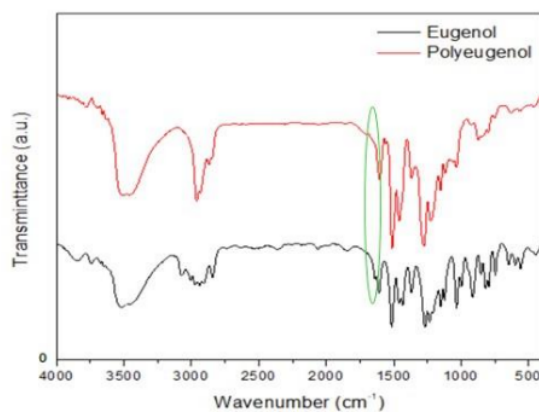


Figure 1. Comparison of FTIR spectra for pure eugenol (black line) and a typical polyeugenol sample (red line).

The antibacterial activity of polyeugenol was compared with eugenol and tested in vitro against pathogenic bacteria *S. aureus* (gram positive) and *E. coli* (gram negative) using disc diffusion method. Antibacterial activity was analysed by measuring the diameter of the clear zone against the test bacteria. Ampicillin was used as a positive control because it had activity against *S. aureus* and *E. coli* by inhibiting wall formation and cell membrane permeability [13]. Dimethyl sulfoxide (DMSO) was used as a negative control because this aprotic polar solvent has capability to dissolve polyeugenol and eugenol compounds as test samples and is a solvent that has no antibacterial properties.

Antibacterial activity test of each sample was carried out in a concentration of 10 mg/mL, while positive control was carried out in a concentration of 0.5 mg/mL. The compounds that have been inoculated into the test medium were then observed at 12 and 24 hours of incubation time to determine the effective time of the ability of polyeugenol to inhibit bacteria.

The antibacterial activity results of polyeugenol and comparative compounds are shown in Table 1 and Fig. 2. Polyeugenol showed a higher sensitivity to gram-positive bacteria (*S. aureus*) compared to gram-negative bacteria (*E. coli*). This is indicated by the value of the inhibition zone in *S. aureus* is greater than that of *E. coli*. The difference in sensitivity between *E. coli* and *S. aureus* against polyeugenol probably caused by differences in cell membrane structure. Gram negative bacteria have an outer membrane while gram-positive bacteria do not [14]. The presence of an outer membrane causes foreign molecules to be unable to diffuse easily through the cell wall. Therefore, gram-negative bacteria are more resistant to polyeugenol than gram-positive bacteria.

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

No.	Compound	Inhibitory zone diameter (mm)			
		<i>E. coli</i>		<i>S. aureus</i>	
		12 h	24 h	12 h	24 h
1	Polyeugenol	0.35	0.25	2.46	2.32
2	Eugenol	1.01	0.93	1.71	1.63
3.	Ampicillin (+)	21.42	20.88	7.61	7.35
4.	DMSO (-)	0	0	0	0

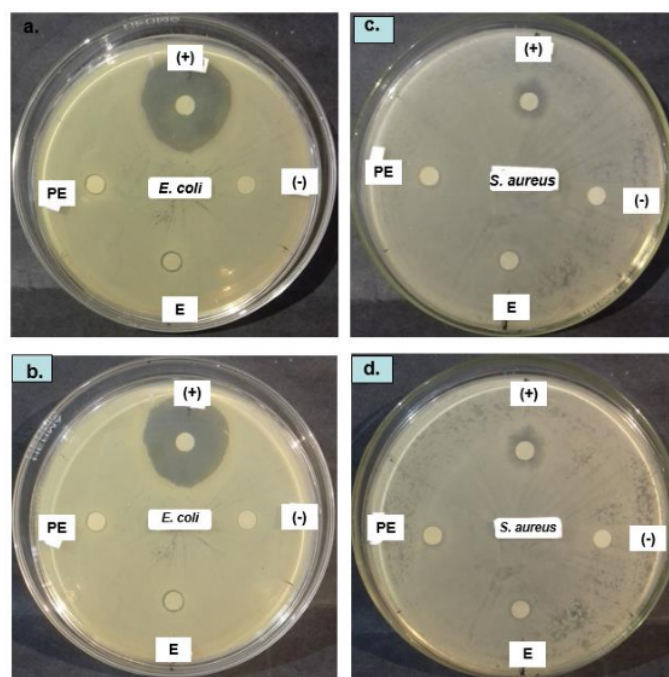


Figure 2. Disc diffusion of antibacterial test against: (a) *E. coli* after 12 h; (b) *E. coli* after 24 h; (c) *S. aureus* after 12 h and (d) *S. aureus* after 24 h (PE = polyeugenol; E = eugenol; (+) = ampicillin; (-) = DMSO).

Inhibition of polyeugenol at a concentration of 20 mg mL^{-1} is known to have lower activity compared to that of eugenol at similar concentration. This is likely because the polymer has a much higher molecular weight than the monomer. Molecules with high molecular weight cannot spread easily on bacterial cell walls compared to low molecular weight compounds. The inhibitory zone value of the polyeugenol was found to be $<3 \text{ mm}$ in both bacteria which categorized as slow response according to Pan *et al.* [15]. Polyeugenol still has antibacterial ability to make this material has the potential to be used in various applications, such as in hygienic applications, textiles, food packaging, and water purification systems.

4. Conclusion

Polyeugenol has been successfully synthesized through a cationic addition polymerization process using BF_3 resulting in an insoluble orange solid in water but in acetone, chloroform, DMSO and benzene solvents. Molecular weight of Polyeugenol using Ubbelohde is 9728 g mol^{-1} to find degree of polymerisation (DP) of 60. Polyeugenol still has antibacterial activity against *E. coli* and *S. aureus* bacteria with a slow response category.

Acknowledgement

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