Synthesis of Quatenary Ammonium Compounds from Eugenol through Mannich and Methylation Reactions and Its Antibacterial Activity

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Abstract: 6-[(N-iodo-N-methyl-N-methyl-N-methylamino)methyl]-4-alyl-2-methoxyphenol was synthesized through Mannich and methylation reaction. Mannich reaction was carried out by reacting eugenol, formaldehyde 37%, and dimethylamine 40% with ethanol at temperature of 78°C for 90 minutes and 4-allyl-6-(dimethylamino)methyl-2-methoxyphenol was obtained with yield of 83,03%. Quartenary ammonium salt, 6-[(N-iodo-N-methyl-N-methyl-N-methylamino)methyl]-4-allyl-2-methoxyphenol, was obtained through methylation reaction of 4-allyl-6-(dimethylamino)methyl-2-methoxyphenol with methyl iodide in ethanol. In the FTIR spectrum, the specific peak of ammonium salt was exhibited at 948.98 and 455.20 cm-1. The antibacterial activity of 6-[(N-iodo-N-metil-N-methyl-N-methylamino)methyl]-4-allyl-2-methoxyphenol was performed against E. coli and S. aureus, this quaternary ammonium salt exhibited a strong activity.

1 INTRODUCTION

Eugenol is the main component contained in clove oil (Syzygium aromaticm), it can reach 70-96%. Therefore, the quality of clove oil is depended on the eugenol content, the increase of eugenol content can influence its quality and price. Eugenol has several functional groups, i.e. hydroxyl (-OH), allyl (-CH_2-CH=CH_2) and methoxy (-OCH_3). These functional groups can be chemically modified to be its derivate which varies bioactivities. Eugenol has been known as an important precursor for synthesizing a particular compound with specific bioactivity (Towaha, 2012).

Many researches that focused in eugenol has been carried out, especially for synthesizing eugenol derivatives, such as the transformation of allyl groups in eugenol into aldehyde groups, that can be found in vanillin which already used as food additives. The other is the transformation of hydroxyl groups into alkyl, acyl or acetyl, that can be found in methyl eugenol, eugenol benzoate and acetyl eugenol (Sastrohamidjojo, 2004).

Amine derivative compounds, such as halide quaternary ammonium salt (R_4N^+ X^-), can be used in wide area. Quaternary ammonium salt can act as a phase transfer catalyst. This catalyst is widely used for heterogeneous reactions involving ionic species in non-polar solvents which cannot dissolve ionic species. Ionic species are commonly found in the liquid phase, while the compounds that will be reacted are found in the organic phase and both species cannot mix each other. The quaternary ammonium salt can also act as a surfactant. Surfactants reduce the surface tension of water by breaking hydrogen bonds on the surface. Most surfactants containing nitrogen bases are cationic surfactants (Perangin-angin, 2002).

Quaternary ammonium salt can be used as an antibacterial (Stanley, 2011). Antibacterial are substances that can inhibit the growth of bacteria by disrupting the microbial metabolism. Antibacterial can only be used if they have selective toxicity, it means they can kill bacteria that cause disease, but they are not toxic to human. The mechanism of antibacterial compounds can be divided into a) inhibiting cell wall synthesis, b) disturbing the cell wall permeability, c) inhibiting the specific enzyme, and d) inhibiting the synthesis of nucleic acids and proteins. Cytoplasm of all living cells are covered by the cytoplasmic membrane that acts as a barrier with selective permeability to carry out as active transport

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arrangement. If the permeability of the cytoplasmic membrane function is disrupted by substances, i.e. surfactant, the permeability of the cell wall will change or even become damaged (Madigan, 2005).

The Mannich reaction is a reaction to modify enolate compounds to be dialkylamino methylene that have active hydrogen atoms. This reaction occurs through a condensation reaction on functionals groups that can be enolized with imminium ions (Mannich base), i.e. a product from the reaction between formaldehyde and secondary amines (Purwono and Daruningsih, 2010). In (Karanov et al., 1995), the synthesis of 2-methoxy-4-(1-prophenyl)-6-phenol through Mannich reaction with eugenol, formaldehyde, and amine compound showed an activities as plant growth control. In the previous work, (Perangin-angin, 2002) performed a synthesis reaction between eugenol and dimethyl sulphate in alkaline condition, and produced 2,2-methylene-bis-6-methoxy-4-(2-propenyl)phenol with yield of 43%. The other study also performed a synthesis of 6-butylaminomethyl and 6-dibutylaminomethyl from eugenol through Mannich reaction with yield of 50 and 78%, respectively. This study also learn the influence of the effect of primary and secondary amines (Hecht, 2014). (Popovici et al., 1999) synthesized several Mannich oxime base derivatives into quaternary ammonium salts. For the example, 1-(2-hydroxy-5-methylpenyl) -3-dialkylamino-1-propanone was quaternized using methyl iodide in tetrahydrofuran (THF) and ethanol at room temperature with yield of 93 and 65%, respectively. (Perangin-angin, 2019) synthesized 4-allyl-6-(hydroxymethyl)-2-methoxyphenol from eugenol through Mannich reactions followed by methylation and substitution reaction.

Therefore, the objective of the present research was to synthesize quaternary ammonium salt that based on eugenol structure through Mannich reaction and followed by methylation reaction. The obtained quaternary ammonium salt showed a moderate antibacterial activity.

2 MATERIALS AND METHODS

2.1 Materials

Equipment: glassware, rotary evaporator, hotplate with stirrer, Fourier Transform Infrared (FT-IR), Gas Chromatography Mass Spectrometer (GC-MS), Spectrophotometer UV-Vis.

Materials: Eugenol, Dimethylamine, Ethanol, Formaldehyde, Methyl iodide, Na_2SO_4 anhydride, Diethyl ether, Nutrient agar (NA), *S. aureus, E. coli*.

2.2 Synthesis of 4-Allyl-6-(Dimethyl amino) Methyl-2-Methoxy Phenol Compound

As much as 4.8 g of eugenol was dissolved with 28 mL of ethanol into the three neck round bottom flask and then 3.8 g (3.48 mL ; 0.04 mol) of formaldehyde (37 wt.%) and 5.6 g (6.3 mL ; 0.05 mol) of dimethylamine (40 wt.%) were followed by reflux process at 78^{o}C for 90 minutes. The mixture was cooled and stirred for 24 h. The excess ethanol was then evaporated by rotary evaporator. The obtained result was characterized by FT-IR and GC-MS.

2.3 Synthesis of 6-[(N-Iodo-N-Methyl-N Methyl-N-Methylamino) Methyl] 4-Allyl-2-Methoxy Phenol

As much as 4.404 g (0.2 mol) of 4-allyl-6-(dimethyl amino)methyl-2-methoxyphenol was added into erlenmeyer. Ethanol (35 mL) and methyl iodide (0.2 mol) were added into erlenmeyer, and tightly closed. The mixture was stirred at room temperature with a magnetic stirrer for 2 h and allowed to stand in the refrigerator for one night. The precipitate formed was filtered using filter paper and washed with diethyl ether. The obtained product was purified by recrystallization process using ethanol and analysed by FT-IR.

2.4 Preparation of Nutrient Agar Slant (NA)

About 7 g of NA was dissolved with 250 mL of distillate water and sterilized in an autoclave at 121^{o}C for 15 minutes.

2.5 Preparation of Medium Agar Slant and Bacterial Culture Stock

The NA slant was prepared by adding 3 mL of NA into test tube and placed it in the rack. Tilt the rack onto solid surface so that the medium is slanted. Allow the medium to harden in this position. The culture was obtained from stock and taken with an osse. This culture was incubated at 35^{o}C for 18-24 h.

2.6 Preparation of Mueller Hinton Agar (MHA) Medium

MHA (19 g) was entered into erlenmeyer and dissolved with 500 mL of distillate water sterilized in an autoclave at 121ºC for 15 minutes.

2.7 Preparation of Bacterial Inoculum

Nutrient broth (3.25 g) was dissolved with 250 mL of distillate water and sterilized in an autoclave at 121ºC for 15 minutes. Furthermore, microbial colony was taken from culture stock using a sterilized osse. The culture was suspended into 10 mL of sterilized nutrient broth in the test tube and incubated at 35ºC for 3 h. The optical density of bacterial was determined using spectrophotometer UV-Vis at 580-600 nm.

2.8 Evaluation of Antibacterial Activity

The antibacterial activity of quaternary ammonium salt was obtained by diffusion method. Paper disk (Ø 6 mm) had been soaked in various concentration of quaternary ammonium salt (10, 20, and 30%). This paper disk then placed on the agar medium that has been cultured with *E. coli* and *S. aureus*. The inhibition zone was measured using calliper (mm).

3 RESULTS AND DISCUSSION

3.1 Synthesis of 4-Allyl-6-(Dimethyl amino) Methyl-2-Methoxy Phenol

The ammonium quaternary salt, 4-Allyl-6-(dimethyl amino)methyl-2-methoxyphenol, was obtained as blackish brown liquid with amount of 5,51 g. The FT-IR spectrum of 4-Allyl-6-(dimethyl amino)methyl-2-methoxyphenol was showed in Figure 1.

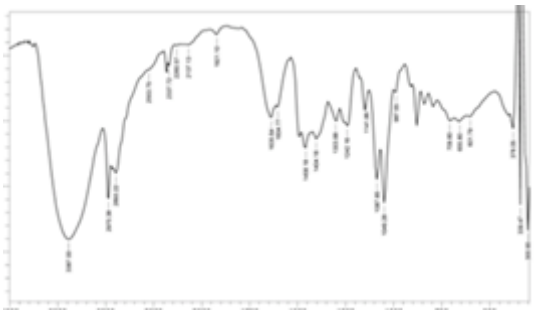

Figure 1: FT-IR spectrum of 4-Allyl-6-(dimethyl amino)methyl-2-methoxyphenol.

The spectrum showed that C-N bonding from dimethylaminomethyl was exhibited at 1246.16 cm^{-1}. The exhibited signal at 3387 cm^{-1} was identified as vibration of OH stretching. The signal at 2970.38 and 2893.22 cm^{-1} showed the vibration of aliphatic CH, this signal was supported by the presence of methylene signal at 1458.18 cm^{-1} and methyl group at 1303.88 cm^{-1}. The presence of C=C aromatic was shown at 1635.64 cm^{-1}. The vinyl group was shown at 987.55 cm^{-1} and C-O-C from ether was shown at 1141.86 cm^{-1}.

4-allyl-6-(dimethyl amino) methyl-2-methoxy phenol was obtained from eugenol through the Mannich reaction, which was reacted with iminium ion. In the Mannich reaction of eugenol, the active hydrogen from eugenol was replaced by the dimethylaminomethyl. The mechanism reaction of this quaternary ammonium salt formation was displayed in Figure 2.

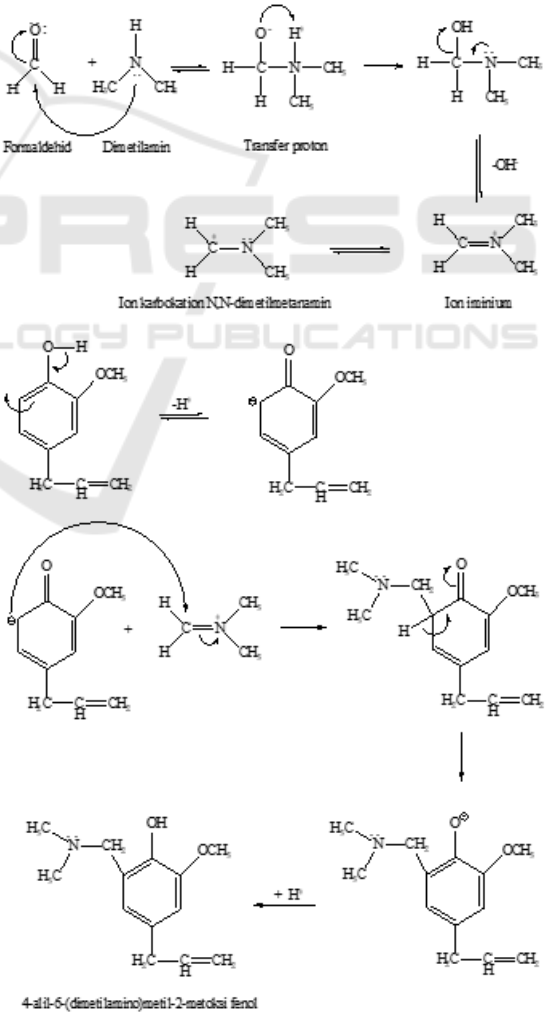

Figure 2: Mechanism reaction of the Mannich eugenol.

The obtained quaternary ammonium salt of 4-allyl-6-(dimethyl amino) methyl-2-methoxyphenol compound using GC-MS showed peak of retention time at 31.428 minute with purity as much 85.65%. The mass chromatogram of the compound synthesized by GC-MS was showed in Figure 3.

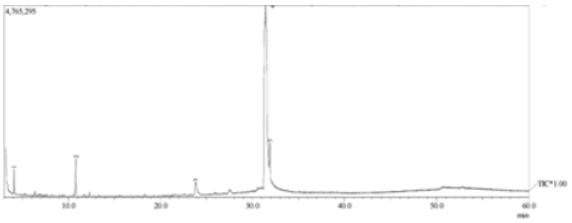

Figure 3: FT-IR spectrum of 4-Allyl-6-(dimethyl amino)methyl-2-methoxyphenol.

The obtained result of 4-Allyl-6-(Dimethylamino)Methyl-2-Methoxy Phenol spectrum was corresponded in Figure 4.

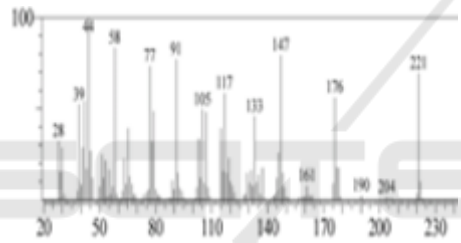

Figure 4: Mass spectrum of 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compound.

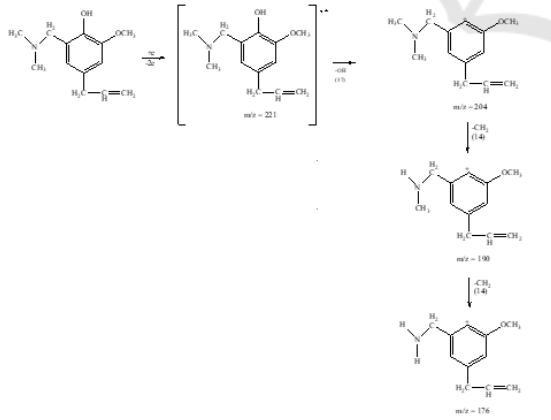

Figure 5: Mass spectrum of 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compound.

The peak of retention time for 31.428 minute with molecular formula $C_{13}H_{19}NO_2$ that had relative molecular mass was 221 g/mol. The Spectral data showed molecular ion peaks at m/e 221 followed by fragmentation peaks at m/e 204, 190, 176, 161, 147, 133, 117, 105, 91, 77, 58, 44, 39, and 28, which was these values corresponded into the relative molecular mass (Mr) of the 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compound synthesized. The fragmentation pattern can be seen in Figure 5.

3.2 Synthesis of 6-[(N-Iodo-N-Methyl-N-Methyl-N-Methylamino) Methyl]-4-Allyl-2-Methoxy Phenol Compound

In this study, the obtained result of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-allyl-2-methoxy phenol was 5.71 in the form of yellow solid and its FT-IR spectroscopy analyzed was showed in Figure 6.

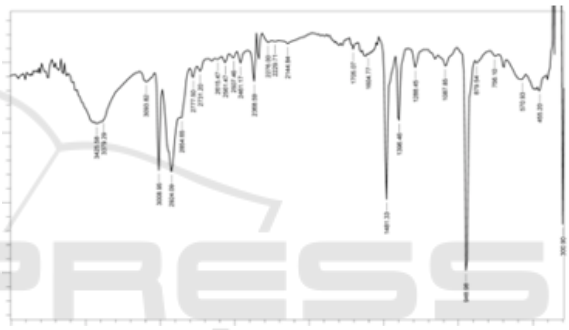

Figure 6: FT-IR spectrum of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-alyl-2-methoxy phenol.

The FT-IR spectrum of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl)]-4-allyl-2-methoxy phenol that can be found stretching vibration at 948.98 cm^{-1} and supported by an absorption at 455,20 cm^{-1}. Those Wavenumbers of 948.98 cm^{-1} to 455.20 cm^{-1} were typical of quaternary ammonium salts that appeared simultaneously. The absorption peak at 3379.29 cm^{-1} was assigned as the O-H vibration after that, the peak at 3008,95 cm^{-1} was corresponded as stretching vibration of C-H sp2 (=CH-) and the wavenumber at 2924,09 cm^{-1} was showed as C-H sp^3 on CH_2. The absorption peak at 1481,33 cm^{-1} indicated CH_2 and C=C aromatic was showed at 1604.77 cm^{-1}. The reaction of preparation 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-allyl-2-methoxy phenol was showed in Figure 7.

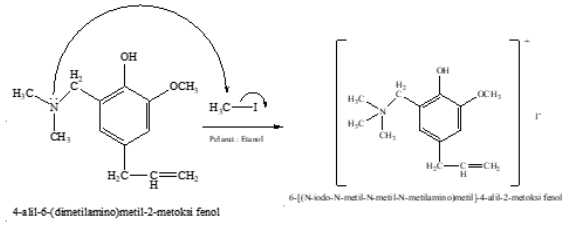

Figure 7: FT-IR spectrum of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-alyl-2-methoxy phenol.

3.3 Antibacterial Activity

Antibacterial activity of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-alyl-2-methoxy phenol using E. coli and S. aureus can be found in Table 1.

Table 1: Antibacterial activity test of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-alyl-2-methoxy phenol.

Treatment	Disc diameter (mm)	*Clear zone diameter (mm)*	
		S. aureus	*E. coli*
10%	6	10.2	10.8
20%	6	10.8	11.4
30%	6	11.3	12.1

Based on antibacterial activity showed that 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-alyl-2-methoxy phenol had antibacterial properties for both of S. aureus and E. coli. It was caused that 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-alyl-2-methoxy phenol had a cationic charge amine group which was able to bind food source of these bacteria that inhibited food nutrition into bacterial cells (Nascimento et al., 2000).

According to (Aleksandra et al., 2017) said that antibacterial activity was classified to be 3 groups. There were strong that produced inhibition zone diameter at 8 mm, medium activity that produced inhibition zone at 7-8 mm, while weak activity that produced inhibition zone diameter less than 7 mm. Therefore, quartenary ammonium compound has strong antibacterial activity.

4 CONCLUSIONS

In this work, synthesis of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-allyl-2-methoxy phenol was performed by 2 step, namely Mannich and Methylation reactions. The obtained result of 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol was 5.51 g with a yield of 83.03% and 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-alyl-2-methoxy phenol was 5.71 g with a yield of 78.39%. The characterization by FT-IR confirmed the existence of 6-[(N-iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-allyl-2-methoxy phenol as quaternary ammonium salt to the stretching vibrations of C-N+ and the peak at 948.98 cm^{-1} and supported by absorption vibration $C\text{-}N^{+}$ at 455.20 cm^{-1}. Furthermore, 6-[(N-Iodo-N-methyl-N-methyl-N-methylamino) methyl]-4-allyl-2-methoxy phenol can be used as antibacteria which showed antibacterial activity for both of *E.coli* and *S. aureus*. This compound showed antibacterial activity for *E. coli* was better compared to *S. aureus* and classified to strong antibacterial activity.

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REFERENCES

Aleksandra, O. B., N, D. Z., Rzepkowska, A., Ko, D., 2017. Comparison of Antibacterial Activity of Lactobacillus plantarum Strains Isolated from Two Different Kinds of Regional Cheeses from Poland : Oscypek and Korycinski Cheese 2017.

Hecht, M., 2014. Synthesis and antimalarial activity of new 5–10.

Karanov, E., Iliev, L., Alexieva, V., Georgiev, G. T., Thang, N. T., Natova, L., 1995. Synthesis and Plant Growth Regulating Activity of Some Novel 2-Methoxy-4-(1-or 2-Propenyl)-6-Substituted Phenols 21, 39–47.

Madigan, M. T., 2005. Brock Biology of Microorganisms, 15th ed. Prentice Hall.

Nascimento, G. G. F., Locatelli, J., Freitas, P. C., Silva, G. L., 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian J. Microbiol. 31, 247–256.

Perangin-angin, S., 2002. Pembuatan Turunan Amina dari Eugenol dan Reaksi Turunan Amina Basa Mannich. Universitas Gajah Mada.

Perangin-angin, S., 2019. JCNaR Phenol Compounds from Eugenol Through Mannich Reaction Followed Methylation with Methyl Iodide and Substitution Using NaOH 01, 75–85.

Popovici, I., Comanită, E., Roman, G., Comanită, B., 1999. Synthesis and reactivity of some mannich bases.

IV. Quaternary ammonium iodides from mannich bases oximes. Acta Chim. Slov. 46, 413–420.
Purwono, B., Daruningsih, E. R. P., 2010. Nucleophilic Substitution Reaction of Cyanide and Methoxyde Ions to Quaternary Mannich Base from Vanillin. Indones. J. Chem. 5, 203–206.
Sastrohamidjojo, H., 2004. Kimia Minyak Atsiri. Gadjah Mada University Press, Yogyakarta.
Stanley, P. H., 2011. pine2011.pdf 403–464.
Towaha, J., 2012. The benefits of cloves eugenol in various industries in Indonesia. J. Perspekt. 11, 79–90.