Synthesis of 6-alyl-8-methoxy-3-propyl-1,3-benzoxazine and 4-alyl-6-(dimethylamino) methyl-2-methoxy phenol from Eugenol through Mannich Reaction and Antibacterial Activity Test

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Keywords: Antibacterial, Eugenol, Mannich Reaction, 4-alyl-6-(dimethylamino)methyl-2-methoxy phenol, 6-alyl-8-methoxy-3-propyl-1,3-benzoxazine

Abstract: 6-alyl-8-methoxy-3-propyl-1,3-benzoxazine and 4-alyl-6- (dimethylamino) methyl-2-methoxy phenol compounds have been synthesized through the Mannich reaction. The 6-alyl-8-methoxy-3-propyl-1,3-benzoxazine compound was synthesized by reacting eugenol, 37% formaldehyde, and propylamine under conditions of reflux with ethanol solvents at 78ºC for 6 hours obtained compounds of 6,116 grams (82.54%). The formation of 6-alyl-8-methoxy-3-propyl-1,3-benzoxazine compound characterized by FT-IR obtained CN stretching vibration at wave number 1242.16 cm-1 and peak molecular ion m/e 247 through GC-MS analysis. The 4-alyl-6- (dimethylamino) methyl-2-methoxy phenol compound was synthesized by reacting eugenol, 37% formaldehyde, and 40% dimethylamine under reflux conditions with ethanol solvents at 78ºC for 90 minutes and obtained compounds of 5,728 grams (86.39%). 4-alyl-6- (dimethylamino) methyl-2-methoxy phenol compound characterized by FT-IR obtained CN and OH stretch vibrations at wave numbers 1242.16 cm-1 and 3410.15 cm-1 and molecular ion peaks m/e 221 through GC-MS analysis. Then the two antibacterial activity tests were carried out on the two compounds using Streptococcus mutans and Escherichia coli bacteria with various concentrations of 10%, 20%, and 30%. The results obtained showed that the 6-alyl-8-methoxy-3-propyl-1,3-benzoxazine and 4-alyl-6- (dimethylamino) methyl-2-methoxy phenol compounds were classified as strong antibacterial.

1 INTRODUCTION

Eugenol is a phenolic compound that has several functional groups such as allyl, hydroxide and methoxy. With the existence of these functional groups, the compound eugenol can be transformed into a number of useful derivative compounds or to be the basic material for the manufacture of other compounds.

Some eugenol derivatives that have been carried out are alkylation, addition, isomerization, acetylation, esterification, polymerization, monoeter cyclization and so on (Suryanto, 2008). Perangin-angin (2019) have synthesized of 4-alil-6-(hidroxymethyl)-2-methoxy phenol compounds from eugenol through Mannich reaction followed methylation with methyl iodide and substitution using NaOH.

Eugenol is a class of phenylpropanoid chemical compounds that have the potential for local anesthetics that have been used medically by dentists. There are phenol functional groups that have antioxidant, anti-inflammatory, anti-allergic, antithrombotic, antimicrobial and antineoplastic activities (Soekardjo, 2000).

This ability is obtained from the lipophilic nature of eugenol which can cause bacterial cell membranes to undergo adhesion which causes inhibited bacterial respiration. This will cause disruption of ion transport in cells so that bacteria experience death. In addition, phenol groups contained in eugenol when attached to bacterial cells will make bacteria undergo lysis, then die (Kumala, 2008).

Karanov et al. (1995) have synthesized eugenol derivatives using formaldehyde and various types of amines through the Mannich reaction. The new compound formed is 2-methoxy-4- (2-propenyl) -6-phenol-substituted aminomethyl derivative at position 6 of eugenol which is known to have activity as a plant growth regulator and pesticide.

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Synthesis of 6-Alyl-8-Methoxy-3-Propyl-1,3-Benzoxazine and 4-Alyl-6-(Dimetilamino)Methyl-2-Methoxy Phenol from Eugenol through Mannich Reaction and Antibacterial Activity Test.
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One example of a compound that has been synthesized is 4-allyl-6- (dimethylamino) methyl-2-methoxyphenol. The compound 4-allyl-6-(dimethylamino) methyl-2-methoxyphenol can be synthesized by reacting eugenol, dimethylamine, and formaldehyde through the Mannich reaction. Mannich reaction is a condensation reaction of ammonia or primary amine or secondary amine and formaldehyde with compounds containing acidic H atoms bound to C or N atoms. In the Mannich reaction, aldehyde condensation with ammonia or primary amines or secondary amines will form the Schiff base as an intermediate product.

The final product of the Mannich reaction is the β-amino-carbonyl compound or the Mannich base (Pine et al., 1988). Rudyanto et al. (2014) synthesized benzoxazine and aminomethyl compounds from eugenol and studied their biological activity. Eugenol is reacted with formaldehyde and methylamine following the Mannich reaction. The benzoxazine compounds obtained are then hydrolyzed to produce aminomethyl derivatives. Furthermore, the benzoxazine and aminomethyl compounds obtained were tested for their biological activity using the Brine Shrimp Lethality Test (BSLT), which is testing the toxicity of a compound against Artemia salina larvae.

Antibacterial activity test can be done by diffusion and dilution methods. Disc diffusion test or disk diffusion test is done by measuring the diameter of the clear zone (clear zone) which is an indication of the inhibitory response of bacterial growth by an antibacterial compound in the extract. Requirements for the number of bacteria for sensitivity test are 105-108 CFU / mL (Hermawan, 2007).

The diffusion method can be done in 3 ways namely the cylinder method, the hole method and the paper disc method. The hole method is to make a hole in a solid agar that has been inoculated with bacteria. The number and location of the holes are adjusted to the purpose of the study, then the holes are injected with the extract to be tested. After incubation, bacterial growth was observed to see the presence or absence of barriers around the hole (Kusmayati, 2007).

Based on the above background, researchers are interested in synthesizing benzoxazine and aminomethyl compounds from eugenol through the Mannich reaction using primary amines and secondary amines to test their antibacterial activity.

2 MATERIALS AND METHOD

2.1 Synthesis of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine Compounds

Into the 100 mL three-neck flask with magnetic stirrer included 28 mL ethanol and 4.8 g (0.03 mol) eugenol were added. After dissolving, 37% formaldehyde was added as much as 4 g (0.05 mol) and 3.6 g (0.06 mol) propylamine followed by reflux at 78ºC for 6 hours. The mixture is cooled and stirred with a magnetic stirrer for 24 hr. Furthermore, the excess ethanol is evaporated with a rotary evaporator. The results obtained were analyzed with FT-IR and GC-MS spectrophotometers.

2.2 Synthesis of 4-allyl-6-(dimethylamino) methyl 2-methoxy phenol Compound

Into the 100 mL three-neck flask with magnetic stirrer included 28 mL ethanol and 4.8 g (0.03 mol) eugenol were added. After dissolving, 37% formaldehyde was added as much as 3.8 g (0.04 mol) and 5.6 g (0.05 mol) dimethylamine 40% followed by reflux at 78ºC for 90 minutes. The mixture is cooled and stirred with a magnetic stirrer for 24 hours. Furthermore, the excess ethanol is evaporated with a rotary evaporator. The results obtained were analyzed with FT-IR and GC-MS spectrophotometers.

2.3 Preparation of Nutrient Agar Slant

About 7 g of NA was dissolved with 250 mL of distillate water and sterilized in an autoclave at 121ºC for 15 minutes.

2.4 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 35ºC for 18-24 h.

2.5 Preparation of Mueller Hinton Agar (MHA) Medium

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

2.6 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 bacterial 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.

2.7 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. mutans. The inhibition zone was measured using calliper (mm).

3 RESULTS AND DISCUSSION

3.1 Synthesis of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine Compounds

Eugenol used in this study is eugenol p.a E'Merck with a purity level of ≥99%. The 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound obtained in the form of a mixture of 6.116 grams (82.54%), in the form of a blackish-brown liquid.

FT-IR spectroscopic data of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound gives a spectrum with vibrational peaks in the region of wave number 3070.68 cm-1; 2947.23 cm-1; 2831.50 cm-1; 1597.06 cm-1; 1458.18 cm-1; 1242.16 cm-1; 1149.57 cm-1; 987.55 cm-1. The results of FT-IR analysis of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compounds can be seen in Figure 1.

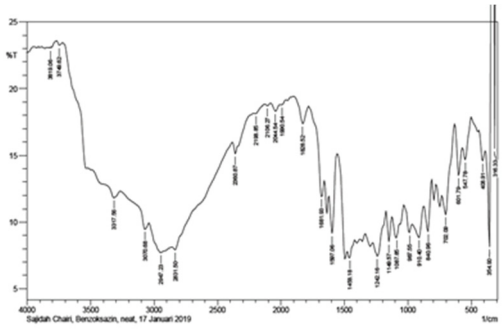


Figure 1: FT-IR of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound.

The spectrum shown from FT-IR data supports that the compound 6-ally-8-methoxy-3-propyl-1,3-benzoxazine has a CN bond originating from the benzoxazine group with the appearance of CN stretching vibrations at wave number 1242,16 cm-1. The absorption peak at wave number 3070.68 cm-1 shows the range of CH sp2, and the area of 2947.23 cm-1 and 2831.50 cm-1 shows the range of CH sp3 of alkyl which is reinforced by the peak at 1458.18 cm-1 for the group methylene (—CH2—) and C = C aromatic are shown at wave number 1597.06 cm-1. The vinyl group is shown at 987.55 cm-1. The peak at 1149.57 cm-1 shows the stretch C-O-C of the ether.

The 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound is obtained from eugenol through the Mannich reaction, where eugenol is reacted with iminium ions which were previously formed through the reaction between formaldehyde and propylamine. In the Mannich reaction of eugenol, the active hydrogen from eugenol is replaced by the propylaminomethyl group as an iminium ion. Then the active nitrogen from the propylaminomethyl group attacks the aluminum ion again to form an oxazine ring by releasing propylamine. The Mannich reaction was carried out under reflux conditions at a temperature of 78 ° C for 6 hours using ethanol solvent. The results of the eugenol, formaldehyde and propylamine reactions produce 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine derivatives.

The mechanism of the 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine reaction can be seen in Figure 2.

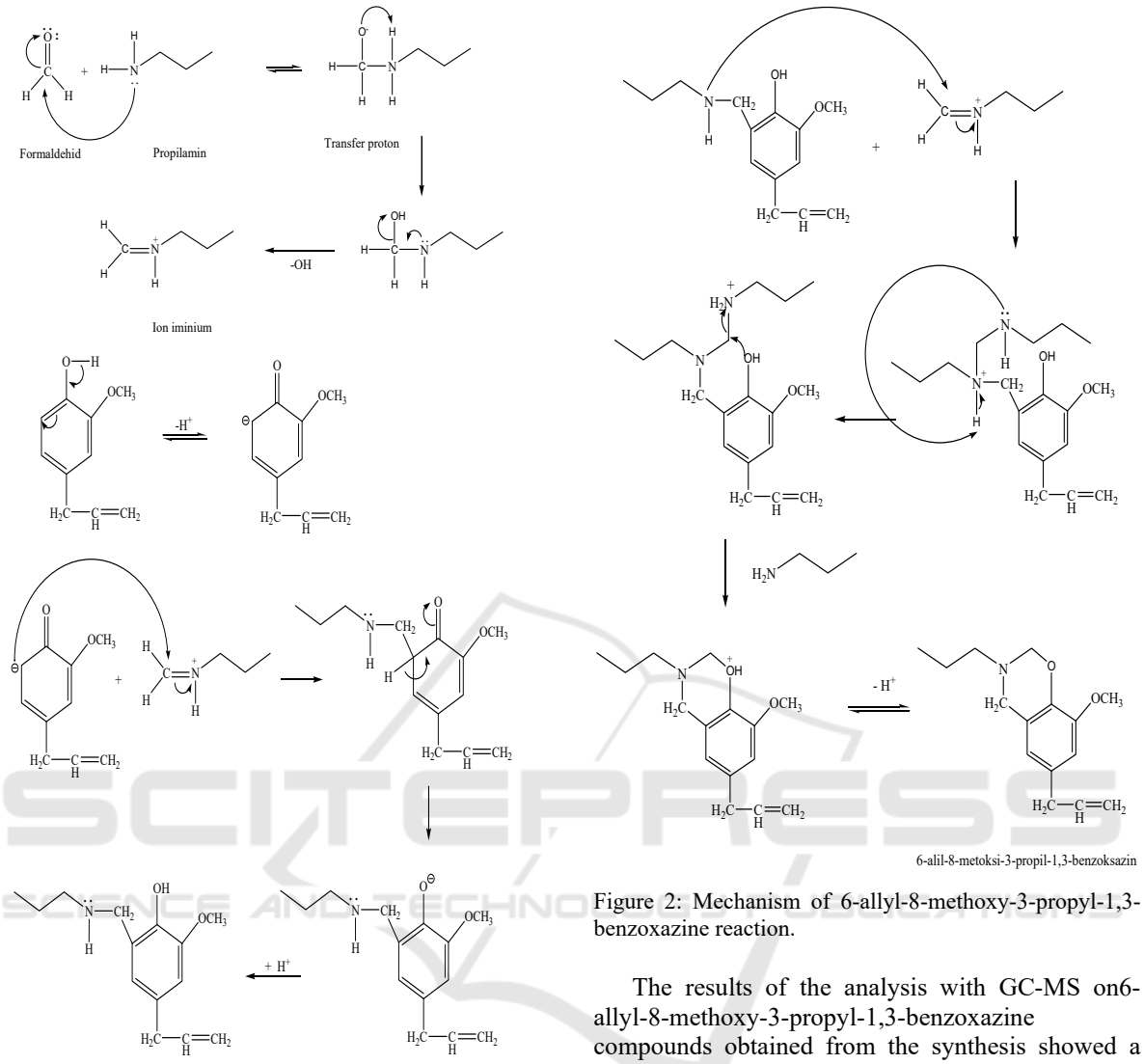
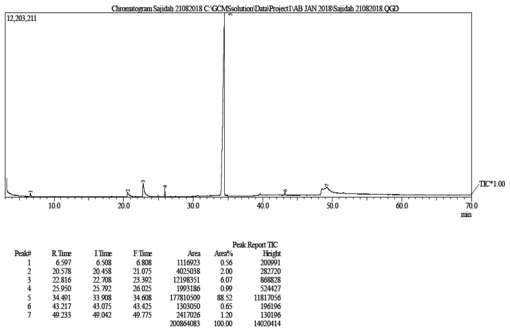


Figure 2: Mechanism of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine reaction.

The results of the analysis with GC-MS on6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compounds obtained from the synthesis showed a peak retention time of 34,491 minutes with a purity of 88.52%. Mass chromatograms of compounds synthesized by GC-MS can be seen in Figure 3.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height
1	6.597	6.508	6.808	1116923	0.56	202091
2	20.578	20.458	21.075	4025038	2.00	282720
3	22.816	22.708	23.392	12198351	6.07	868828
4	25.950	25.792	26.025	1993186	0.99	324427
5	34.491	33.908	34.608	177810509	88.52	11817056
6	43.217	43.075	43.425	1303050	0.65	190196
7	49.233	49.042	49.775	2417026	1.20	130196
				200864083	100.00	14020414

Figure 3: GC-MS spectra of identified compounds.

The spectrum of compound 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine obtained is shown in Figure 4.

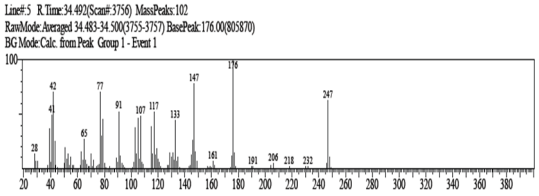


Figure 4: Peak detection of identified compounds.

The peak with a retention time of 34.491 minutes is a compound with the molecular formula $C_{15}H_{21}NO_2$ with a relative molecular mass of 247 g / mol. Spectrum data show molecular ion peaks at m / e 247 followed by fragmentation peaks at m / e 232, 218, 206, 191, 176, 161, 147, 133, 117, 107, 91, 77, 65, 42, 41, and 28 where this value corresponds to the relative molecular weight (Mr) of the synthesized 6-ally-8-methoxy-3-propyl-1,3-benzoxazine compound. Fragmentation patterns can be seen in Figure 5.

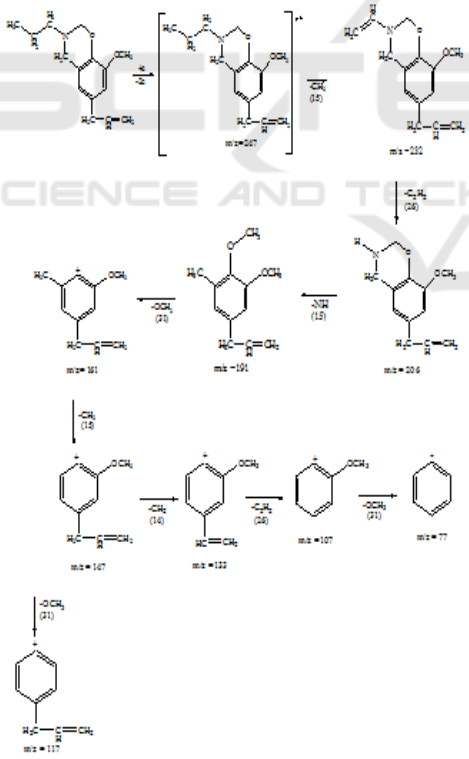


Figure 5: Fragmentation pattern of synthesized compound.

3.2 Synthesis of 4-allyl-6-(dimethylamino) compound methyl-2-methoxy phenol

Eugenol used in this study is eugenol p.a E'Merck with a purity level of ≥99%. Compound 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol obtained in the form of a mixture of as much as 5.728 grams (86.39%), in the form of a blackish brown liquid.

FT-IR spectroscopy data of 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compound gives a spectrum with vibrational peaks in the region of wave number 3410.15 cm-1; 3070.68 cm-1; 2947.23 cm-1; 2900.94 cm-1; 2831.50 cm-1; 1597.06 cm-1; 1465.90 cm-1; 1242.16 cm-1; 1149.57 cm-1; 987.55 cm-1. The results of FT-IR analysis of 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compounds can be seen in Figure 6.

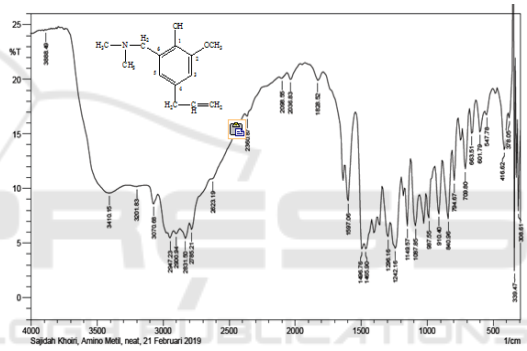


Figure 6: FT-IR of 4-allyl-6- (dimethylamino) methyl-2-methoxy phenol compound.

The spectrum shown from FT-IR data supports that the 4-allyl-6- (dimethylamino) compound methyl-2-methoxy phenol formed has a CN bond originating from the dimethylaminomethyl group with the emergence of CN stretching vibrations at the wave number 1242.16 cm- 1 The absorption peak at wave number 3410.15 cm-1 shows the O-H vibrations. The absorption peak at wave number 3070.68 cm-1 shows the range of C-H sp2 and in the area of 2947.23 cm-1; 2900.94 cm-1 and 2831.50 cm-1 shows the CH sp3 range of alkyl reinforced with a peak at 1465.90 cm-1 for the methylene group (—CH2—) and C = C aromatic shown at wave number 1597.06 cm-1. The vinyl group is shown at 987.55 cm-1. The tape at 1149.57 cm-1 shows the stretch C-O-C of the ether.

The 4-allyl-6- (dimethylamino) methyl-2-methoxy phenol compound is obtained from eugenol through the Mannich reaction, where eugenol is reacted with iminium ions which were previously

formed through the reaction between formaldehyde and dimethylamine. In the Mannich reaction, the active hydrogen from eugenol is replaced by the dimethylaminomethyl group. The Mannich reaction was carried out under reflux conditions at a temperature of 78°C for 90 minutes using ethanol as a solvent. The results of the eugenol, formaldehyde and dimethlamine reactions produce the 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol derivative.

The reaction mechanism of the 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compound can be seen in Figure 7.

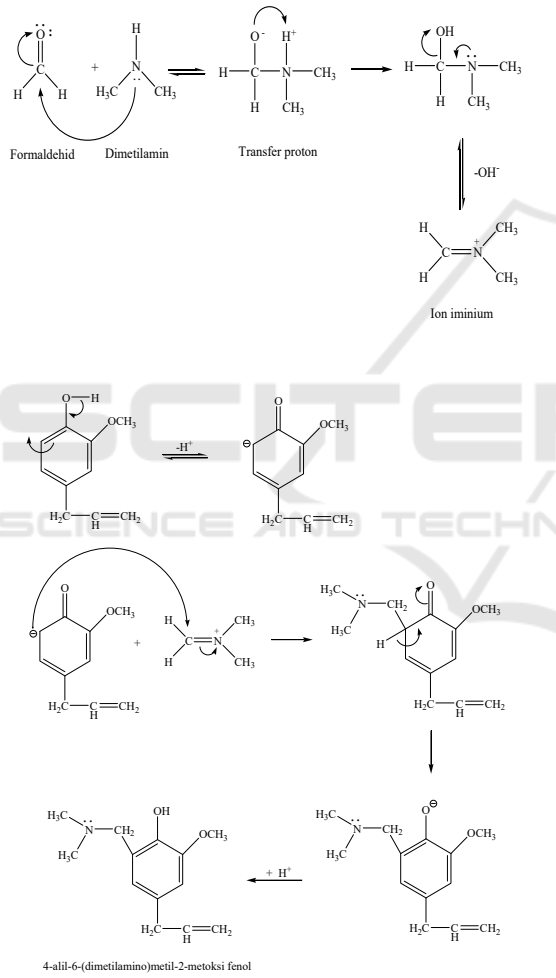


Figure 7: Reaction mechanism of the 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol.

Results of analysis with GC-MS on 4-allyl-6-(dimethylamnino) methyl-2-methoxy phenol compounds obtained from the synthesis showed a peak retention time of 29,650 minutes with a purity of 86.54%. Mass chromatograms of compounds synthesized by GC-MS are shown in Figure 3.8.

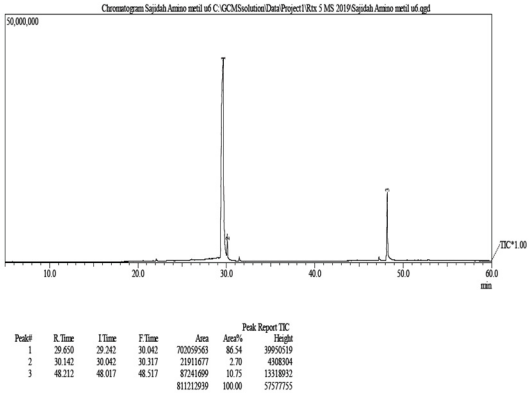


Figure 8: GC-MS spectra of identified compounds.

The spectrum of the compound 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol obtained was shown in Figure 9.

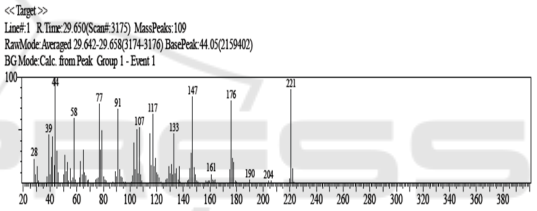


Figure 9: Peak detection of identified compounds.

The peak with a retention time of 29,650 minutes is a compound with the molecular formula $C_{13}H_{19}NO_2$ with a relative molecular mass of 221 g / mol. Spectrum data show the peaks of molecular ions at m / e 221 followed by fragmentation peaks at m / e 204, 190, 176, 161, 147, 133, 117, 107, 91, 77, 58, 44, 39, and 28, where this value corresponds to the relative molecular weight (Mr) of the 4-allyl-6-(dimethylamino) compound synthesized methyl-2-methoxy phenol. Fragmentation patterns can be seen in Figure 10.

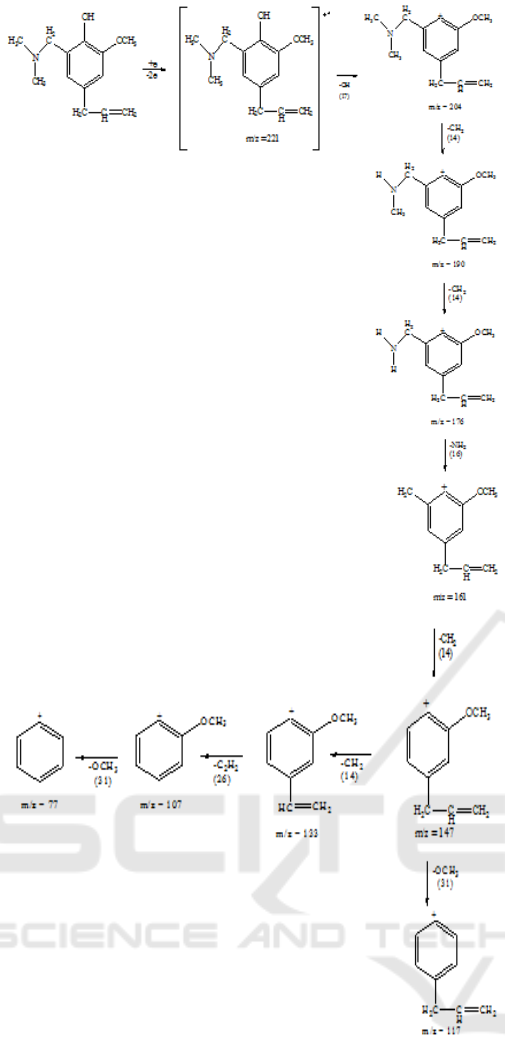


Figure 10: Fragmentation pattern of 4-allyl-6-(dimethylamino) compound.

3.3 Antibacterial Activity Test

Antibacterial activity test of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound and 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compound from the synthesis results in the form of mixture using Streptococcus mutans and Eschericia coli can be seen in Table 1 and 2.

Table 1: Tests for antibacterial activity against 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compounds.

Treatment	Disc Diameter (mm)	Clear Zone Diameter (mm)	
		S. mutans	*E. coli*
10%	6	18.5	21
20%	6	33	21
30%	6	33	14

Table 2: Tests for antibacterial activity against 4-allyl-6-(dimethylamino) methyl-2-methoxy phenol compounds.

Treatment	Disc Diameter (mm)	Clear Zone Diameter (mm)	
		S. mutans	*E. coli*
10%	6	12	18
20%	6	19	16
30%	6	17	14

Data on antibacterial activity test results showed that there were antibacterial activities for both *Streptococcus mutans* and *Escherichia coli* bacteria in 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine and 4-allyl-6- (dimethylamino) methyl-compound 2-methoxy phenol. This is because the 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound and the 4-allyl-6- (dimethylamino) methyl-2-methoxy phenol compound have a nitrogenous base group. This base group will react with amino acids that make up the cell wall and bacterial DNA which are the main constituents of the cell nucleus. This reaction results in changes in the structure and composition of amino acids that cause changes in genetic balance so that the bacterial DNA will be damaged. Cell damage to bacteria will eventually make the bacteria unable to metabolize so that it will also undergo lysis. Thus, the bacteria will become inactive and destroyed (Gunawan, 2008).

In the antibacterial activity test of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound against *Streptococcus mutans*, the antibacterial activity is stronger than eugenol. This is shown by the highest clear zone diameter at concentrations of 20% and 30%, which is 33 mm while in eugenol the highest clear zone diameter is 18 mm at a concentration of 10%.

In the antibacterial activity test of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound against *Escherichia coli*, the highest clear zone diameter at concentrations of 10% and 20% is 21 mm and has decreased at a concentration of 30% that is equal to 14 mm. In the antibacterial activity test of 4-allyl-6- (dimethilamino) methyl-2-methoxy phenol compound against *Streptococcus mutans* the highest clear zone diameter was obtained at a concentration of 20% at 19 mm and decreased at a concentration of 30% at 17 mm, whereas, against Escherichia coli the highest clear zone diameter at a concentration of 10% is 18 mm and has decreased at 20% and 30% that is 16 mm and 14 mm. Based on the test results of antibacterial activity against bacteria *Streptococcus mutans* and *Escherichia coli* obtained inhibitory zone diameters

that have fluctuated and not proportional to the concentration of the compound.

Elifah (2010) suggested that the diameter of the inhibition zone does not always increase in proportion to the increase in antibacterial concentration. This can occur because of differences in the speed of diffusion of antibacterial compounds on agar media. Different types and concentrations of antibacterial compounds give different inhibitory zone diameters for a certain period of time. Irregularity in the diameter of the zone of inhibition of the growth of test bacteria is at the time of unequal disk drying. Therefore, it causes a inhibitory zone at the highest concentration to decrease. Disk which has long drying time, when it is placed on top of the bacterial hatching media, the area of the inhibitory zone is small, this zone is formed from extracts diffused from the disk to the agar media. On disks with only a short drying time, when placed on top of the bacterial hatchery media, the extract which is still attached immediately spreads around the disk and quickly diffuses to the media so as to form a larger inhibitory zone.

Sinarsih (2016) suggests that the presence of unstable antibacterial performance at high concentrations is likely due to compounds in general having a limited ability in bioactivity. So that at increasing concentrations certain compounds do not provide a significant increase in response or not significantly different.

The strength of antibacterial activity can be seen from the inhibitory zone formed. 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. Thus 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound and 4-allyl-6- (dimethylamino) methyl-2-methoxy phenol compounds have relatively strong antibacterial activity.

4 CONCLUSION

Synthesis of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound obtained from eugenol through the Mannich reaction, where eugenol is reacted with iminium ions which were previously formed through the reaction between formaldehyde and propylamine. The results obtained were 6.116 grams (82.54%) compound of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine. The formation of 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound is characterized by the appearance of C-N stretching vibrations at wave number 1242.16 cm-1

Synthesis of 4-allyl-6- (dimethylamino) methyl-2-methoxy phenol compound obtained from eugenol through the Mannich reaction, where eugenol is reacted with iminium ions which were previously formed through the reaction between formaldehyde and dimethylamine. The results obtained were 5,728 grams (86.39%) of the 4-allyl-6- (dimethylamino) compound methyl-2-methoxy phenol. The formation of 4-allyl-6- (dimethylamino) compound methyl-2-methoxy phenol is characterized by the appearance of C-N stretching vibrations at wave number 1242.16 cm-1 and O-H vibrations at wave number 3410.15 cm-1

The 6-allyl-8-methoxy-3-propyl-1,3-benzoxazine compound and the 4-allyl-6- (dimethylamino) methyl-2-methoxy phenol compound exhibit antibacterial activity that is classified as strong against S. mutans and E bacteria .coli. This is indicated by the diameter of the clear zone produced which is more than 8 mm so that both compounds act as antibacterials that are classified as strong.

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