

Conjugation Reaction between Citronellal and L-Tyrosine and Its Antimicrobial Properties against Bacteria and Fungi

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Abstract: The study of citronellal with L-tyrosin conjugation for antimicrobial properties has been conducted. The aim of this study to determine the relationship structure between two compounds citronellal and L-tyrosine on antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The conjugation product obtained was yellow-white solid amorphous with the Rf value was 0.84 and the percentage of yield was 71.12%. The FT-IR spectra peak at 3205.69 cm⁻¹ is represented the N-H stretching vibration from L-tyrosine, while the spectra appears at 1460.11 - 1438.90 cm⁻¹ are represented the C=N which derived from imine or immonium from shift base reaction between citronellal and L-tyrosine. The GC-MS analysis showed that the peak 15 observed at RT 10.27 min. be expected a conjugation product with the m/z 316 [M+H]⁺ ion. The antimicrobial activity were determined by well diffusion method and the results showed that product of conjugation were have no antimicrobail activities at concentration tested.

1 INTRODUCTION

Citronellal is a monoterpene that has two optical isomers with a molecular weight of 154.25 g / mol. The reactivity of citronellal is resulting from carbonyl group, double bond, and acidity of H α . These groups allowing citronellal to react with an acid or a base. Some biological activities of citronellal including insecticides (Griffith and Grentile, 1979), perfumery (Anderson et al., 1993; Sangwan et al., 2001), stimulants, antidepressants, analgesics, antipyretics, and antimicrobials (Adhikari et al., 2015) L-tyrosine or 4-hydroxyphenylalanine is a non-essential amino acid, a primary amine, has a polar group. L-tyrosine widely used in food industry and pharmaceutical

industry (Tina, 2007). The conjugation of natural product with several constituents has attracted some researchers due to their biological reactivity on several microorganisms and cells (Martinez et al., 2015; Hong et al., 2017). For examples, novobiocin, serrulatane, xanthorhizol (Finland and Nichols, 1957; Lewis and Klivanov, 2005; Rukayadi and Hwang, 2006), which contain prenil and aromatic hydroxy groups are believed to play an important role in antimicrobial activity.

2,4-Dimethyl-2,6-heptadiene-1-ol and 5-Amino-2-methylphenol are two compounds produced from a conjugation reaction are known to have antibacterial activity against *Staphylococcus epidermidis* (Ys, 2015), Rusdin reported that product conjugation between citronellal and L-tyrosine has antibacterial

activity against *Staphylococcus aureus* (Al-Garawi et al., 2012). The computational analysis result showed the formation of imine conjugation bonds (imine bond formation) between the citronellal (3,7-dimethyl-6-octenal) with the amino acid L-tyrosine (Rusdin et al., 2018). However, the research conducted by Rusdin and Hardi has not been able to determine the type of conjugate product. In this study, we interest to determine product of conjugation obtained by GC-MS spectroscopy and evaluate its activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

2 EXPERIMENTAL

2.1 Materials

Citronellal and L-tyrosine were received from Department of Chemistry, Universitas Tadulako, Palu, Sulawesi Tengah, Indonesia. Pentane hexane, ethyl acetate, methanol, potassium hydroxide (KOH), diethyl ether, 96% ethanol, distilled water, physiological NaCl, and dimethyl sulfoxide (DMSO) were obtained from Department of Pharmacy, Universitas Syiah Kuala, Banda Aceh, Indonesia.

2.2 Conjugation Reaction Citronellal and L-Tyrosine

The conjugation reaction between citronellal and L-tyrosine was conducted by Al-Gharawi and Rusdin methods (Al-Gharawi et al., 2012; Rusdin et al., 2018). Briefly, 0.18 grams of citronellal (1.2 mmol) in 10 mL of methanol was reacted with 0.18 grams of L-tyrosine (1 mmol) in 15 mL of methanol, then 0.056 grams of KOH was added. The mixture then refluxed for 8 hours at 60°C. The conjugate (product) then concentrated using rotary evaporator and washed three times with pure ethanol. The product washed again with diethyl ether and evaporated at room temperature to obtain yellow-white solid amorphous.

2.3 Column Chromatography

Purification of conjugation product obtained was conducted by column chromatography using silica gel F₂₅₀ as stationary phase. As mobile phase, we used a mixture of hexane: ethyl acetate (9:1). The collected fractions were submitted into thin-layer chromatography (TLC), using mobile phase a mixture of hexane: ethyl acetate (9:1), and the chromatogram was observed using UV-lamp at 250 nm. The major

fraction obtained then analysis by GC-MS spectroscopy.

2.4 FT-IR Analysis

The FT-IR analysis spectrophotometer was performed at wave numbers 4000-500 cm⁻¹ using Shimadzu model.

2.5 GC-MS Analysis

The conjugation product and collected fraction were analysed by gas chromatography-mass spectroscopy 6890 equipped with capillary column Agilent HP 5 MS (60 x 0.25 x 0.25). The operating condition of the gas chromatography were 1.0 ml/min (He), with volume injection was 0.5 µl. Oven temperature 300°C for 40 min.

2.6 Antimicrobial Activity

The antifungal activity was determined by Kirby-Bauer method. The sterile Sabouraud's Dextrose Agar (SDA) media was poured into petri dish and allowed to solidify. The strains of *Candida albicans* was spread out on the solidified media of SDA by using the sterile cotton bud. The paper disc was laid out on the surface of the agar medium. To each of disc 12 µl of negative control (solvent), positive control (nystatine), and tested compound and was loaded and subsequently incubated at 37°C for 48 hours. In the same procedure, the antibacterial activity of the tested compounds against *Staphylococcus aureus* and *Escherichia coli* were performed using Mueller Hinton Agar (MHA) media and subsequently incubated at 37°C for 24 and 48 hours. In the antibacterial assay we used ciprofloxacin and gentamycin as positive controls for *Staphylococcus aureus* and *Escherichia coli* respectively. Then, the inhibition effect of the tested compounds were determined. The antimicrobial activities were performed in triplicates.

3 RESULTS AND DISCUSSION

3.1 Conjugation Reaction Citronellal and L-Tyrosine

Conjugation reaction between citronellal and L-tyrosine were used potassium hydroxide (KOH) as a catalyst. Ritter mentioned that carbonyl group, double bond, and H_α atom from citronellal allows to react with an acid or base. L-tyrosine is a primary amine, a

base, is readily react to citronellal (an aldehyde) (Griffith and Grentile, 1979). Murray mentioned that the reaction between an aldehyde or a ketone with a primary amine will produce an imine through a shift base mechanism. L-tyrosine act as a nucleophilic, and a citronellal act as electrophilic to form an immonium salt, through the mechanism of base shift reaction, this readily to form an imine compound ($R_2C=NR$) (Murray, 2010). The mechanism reaction of imine formation from primary amine and an aldehyde shown in Figure 1 below.

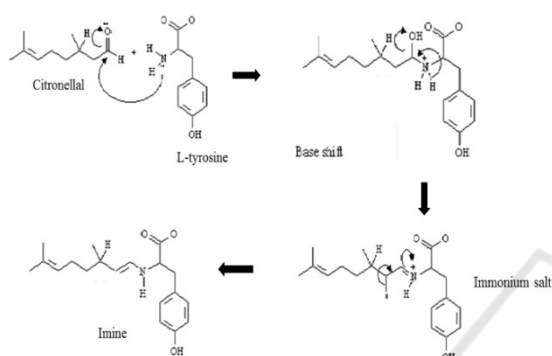


Figure 1: The mechanism reaction of imine formation from citronellal and L-tyrosine.

The obtained product was white-yellow in colour and amorphous in shape. The fragrant of the product was lighter than citronellal. The percentage yield of product was 71.12% or 0.51 gram. The TLC result showed that the product has four bands with the R_f values were 0.17; 0.28; 0.44; and 0.84 (Figure 2A).

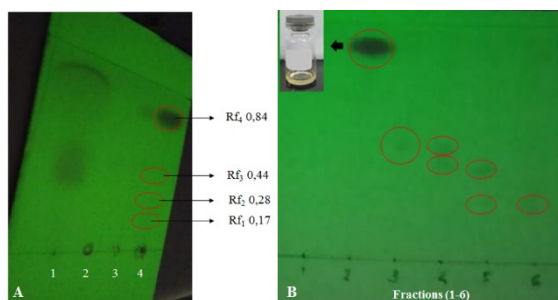


Figure 2A: TLC result of the conjugation product: 1. Citronellal; 2. L-tyrosine; 3. Citronellal + L-tyrosine; and 4. Conjugation product; B. TLC result of the conjugation product from column chromatography using mobile phase a mixture of hexane: ethyl acetate (9:1), and the chromatogram of the fractions was observed using UV-lamp at 250 nm.

3.2 FT-IR Analysis

The FT-IR analysis of citronellal, L-tyrosine, and conjugation product shown below. Figure 3A showed that the spectra at 1729 cm^{-1} indicated the presence of carbonyl groups ($-C=O$), while the absorption at 2913 cm^{-1} and 1423 cm^{-1} were represented of functional group of C-H and C=N. All these functional group are typical for citronellal. Fig. 3B pointed that the absorption at 1589 cm^{-1} , 3363 cm^{-1} and 1242 cm^{-1} were indicated the presence of aromatic functional group of C=C, N-H, and C-N respectively. These functional groups are typical for L-tyrosine.

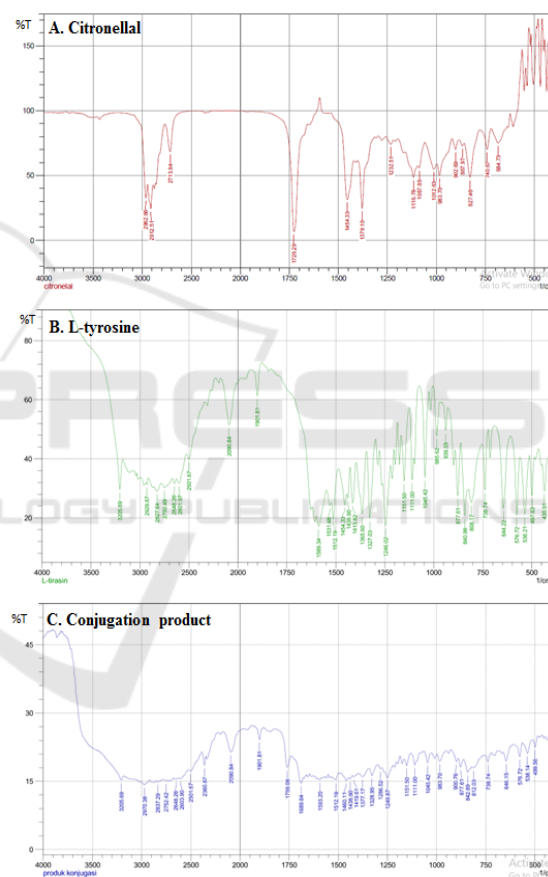


Figure 3: The FT-IR analysis of A. Citronellal; B. L-tyrosine; C. Conjugation product.

The FT-IR analysis of the conjugation product showed strong absorption of N-H groups at 3205.69 cm^{-1} (Figure 3C). Murray and Silverstain mentioned that the absorption of $C=O$ carbonyl appears at 1759.08 cm^{-1} with low intensity. The absorption of $C=C$ alkenes appears at 1669.64 cm^{-1} and the spectrum of $C=C$ aromatic absorb at 1512.19 cm^{-1} .

While the C=N spectrum appears at 1460.11-1438.90 cm^{-1} (Murray, 2010; Silverstein et al., 2005).

3.3 GC-MS Analysis

The major fraction (fraction 2, Figure 2B) obtained from column chromatography with the Rf value of 0.86 that to be expected conjugation product was analysis by GC-MS. The GC spectral of fraction 2 is presented in Figure 4.

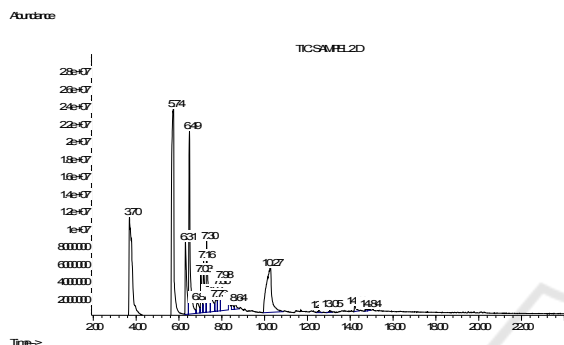


Figure 4: The GC analysis of fraction 2 isolated from conjugation product.

The composition of fraction 2 isolated from conjugation product is shown in Table 2. The profile of fraction 2 from conjugation product contains 20 compounds. The main compound of the fraction 2 is phenolic compound. The major of phenolic compounds in fraction 2 was *p*-cresol (13.20%) and L-Tyrosine and Octanal,7-hydroxy-3,7-dimethyl (12.92%). The other phenolic compounds in moderate percentage were assigned to 3-propyl-phenol (2.3%), 3-ethyl-phenol (2.49%), 2,4-dimethylphenol (4.14%), 4-ethyl-2-methyl-phenol (4.53%), 4-ethylphenol (5.99%), and Octanal,7-hydroxy-3,7-dimethyl (0.53%).

The Ritter stated that L-tyrosine (a primary amine) is readily react to citronellal (an aldehyde) to form an imine compound (conjugation product) through a shift base mechanism (Figure 1) (Griffith and Grentile, 1979). The characterization of the product conducted by GC-MS, to identify the conjugation product between citronellal and L-tyrosine. The MS characterization of the product is shown in Figure 5, and be expected observed at RT 10.27 with the percentage area of the peak of 12.92% (peak 15).

Table 1: The GC-MS analysis of fraction 2 isolated from conjugation product.

No.	Name	RT (min)	% Area	m/z
1.	Carboxylic acid	3.70	16.47	44.0
2.	Phenol	5.74	25.35	94.1
3.	Hydroxytoluene	6.31	5.34	108.14
4.	<i>p</i> -cresol	6.49	13.20	108.14
5.	2,3-dimetyl-phenol	6.87	1.44	122.16
6.	3-ethyl-phenol	7.03	2.49	122.16
7.	2,4-dimethylphenol	7.15	4.14	122.16
8.	4-ethylphenol	7.30	5.99	122.16
9.	3-propyl-Phenol	7.50	2.30	136.19
10.	2-propyl-Phenol	7.73	1.19	139.19
11.	2-ethyl-4-methyl-Phenol	7.86	2.27	137.01
12.	4-ethyl-2-methyl-Phenol	7.97	4.53	137.01
13.	2,3-Dimethyl-N-phenylalanine	8.50	0.49	197.00
14.	Octanal,7-hydroxy-3,7-dimethyl	8.63	0.57	154.00
15.	L-Tyrosine and Octanal,7-hydroxy-3,7-dimethyl	10.27	12.92	316.00
16.	Benzophenone	12.54	0.24	182.00
17.	Phenol, 4-(2-aminoethyl)	13.05	0.18	137.00
18.	2,2-methylenediphenol	14.21	0.57	200.23
19.	4,4'-methylenediacetamide	14.76	0.16	282.33
20.	Tyramine	14.85	0.15	181.00
	Total		100.00	

From the result, the product (peak 15), lose a common molecule of C=O (-28 Da) forming the conjugation product with the m/z 316 $[M+H]^+$ ion. Figure 5, also showed that the fragment of m/z 154 $[M+H]^+$ and m/z 181 $[M+H]^+$ were characteristic for citronellal ($\text{C}_{10}\text{H}_{18}\text{O}$) and L-tyrosine ($\text{C}_9\text{H}_{11}\text{NO}_3$) respectively. However, these results need to be further analysed to ensure that the product formed is a true conjugate product (imine compound).

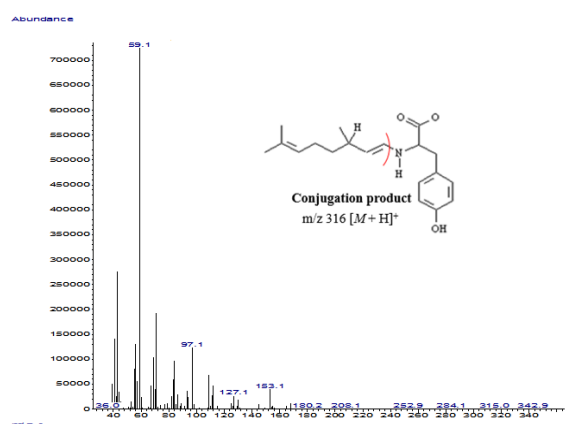


Figure 5: The GC-MS analysis of fraction 2 isolated from conjugation product (peak 15) at RT 10.27.

3.4 Antimicrobial Activity

The antifungal activity of the conjugation product against *Candida albicans* is presented in Figure 6C. The results showed that the conjugation product at concentration of 10 and 50 mg/ml has no activity against *Candida albicans*. While, the positive control (nystatin) showed antifungal activity with the diameter of inhibition zone was 10.70 mm (Table 2).

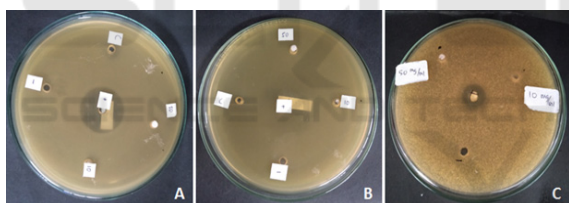


Figure 6: Antimicrobial activity of the conjugation product. A. against *S. aureus*; B. *E. coli*; and C. *C. albicans*.

Table 2: The inhibition effect of the conjugation product against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

Sample		Diameter of inhibition zone (mm)		
		<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
C ⁻	Solvent	nie	nie	nie ^a
C ⁺	Nystatin	nd ^b	nd	10.70
	Ciprofloxacin	21.78	nd	nd
	Gentamycin	nd	29.60	nd
	Citronellal	8.51	nie	nie
Product	10 mg/ml	nie	nie	nie
	50 mg/ml	nie	nie	nie

Note: C⁻: negative control; C⁺: positive control; nie: no inhibition effect; nd: not determined.

The antibacterial activity of the conjugation product also tested, and the results exhibited that the

product has no antibacterial activities on *Staphylococcus aureus* and *Escherichia coli* at concentrations used. In this assay, we used ciprofloxacin and gentamycin as positive controls for *Staphylococcus aureus* and *Escherichia coli* respectively (Table 2).

The results also showed that, ciprofloxacin, gentamycin and citronellal (as precursor for synthesis of conjugation product) have antibacterial activities with the diameter inhibition zone were 21.78; 29.60; and 8.51 mm respectively (Figure 6B and 6C). In the conclusion, the antimicrobial assays showed that the conjugation product have no inhibition effect against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* at concentration tested.

4 CONCLUSION

The conjugated product between citronellal and L-tyrosine produces a powdery and yellowish-white product, with percentage of yield was 71.12%. The TLC analysis showed that a band with the R_f value of 0.85 was thought to be the conjugation product. The GC-MS analysis showed that the fragment ion at m/z 316 [M+H]⁺, lose a common molecule of C=O (-28 Da), was expected to be conjugation product. The antimicrobial assays showed that the conjugation product at the concentrations of 10 and 50 mg/ml have no inhibition effect on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

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