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by Erwin Indriyanti

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A GREEN SYNTHESIS OF N-OCTYL CINNAMATE BY SONOCHEMICAL METHOD AND POTENTIAL AS ANTIOXIDANT AND ANTIINFLAMMATORY IN-VITRO

ErlitaVerdia Mutiara¹, Achmad Wildan², Yustisia Dian Advistasari³,
Erwin Indriyanti⁴, Mighfar Syukur^{5*}

^{1,3,4,5}Department of Pharmacy, Stifar Foundation Pharmacy Semarang

²Department of Pharmaceutical and Food Analysis, Stifar Foundation Pharmacy Semarang

Corresponding Author:email:syukurmighfar@gmail.com

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Abstract: Cinnamic acid and its derivatives have a vital role in the synthesis of other important compounds and as precursors for the synthesis of cinnamic esters. Cinnamic acid and its derivatives have diverse bioactivity. This compound is able to inhibit the grow process from several species of bacteria and fungi. In addition, this compound has antioxidant, hepatoprotective, anti-inflammatory, anxiolytic, insect repellent, antidiabetic and anticholesterolemic activities. One of the cinnamic acid derivative compounds is n-octyl cinnamate. N-octyl cinnamate is a one of the cinnamic acid derivative produce from esterification reaction between cinnamic acid and n-octanol with a concentrated sulfuric acid catalyst. The sonochemical method is one of the methods developed in this synthesis process, because it is very easy to do, efficient, with high percent yields, short time and environmentally friendly. From the results of synthesis, identification of the compound was carried out by means of melting point and FTIR and NMR spectrophotometry. Based on the interpretation data from FTIR, ¹H-NMR and ¹³C-NMR show the result of the synthesis is n-octyl cinnamate. The largest percent yield is produced in 7 hours at temperature 60^o C with 7 hours a sonication time. The activities of antioxidant that show as EC₅₀ value from synthesis n-octyl cinnamate compound was 34.71 g/mL. In anti-inflammatory, n-octyl cinnamate compounds at 100 concentration ppm get % inhibition of 55.56%.

Keywords:Anti-inflammatory, antioxidant, n-octyl cinnamate, Synthesis, Sonochemistry.

I. INTRODUCTOION

Cinnamic acid is found in almost all green plants, even in small populations^[1]. Cinnamic acid is a key between shikimate and phenylpropanoid ways. Shikimate acid is a precursor of many alkaloids, aromatic amino acids, and indole derivatives. It is found in free form, and especially in ester form (ethyl, cinnamyl, benzyl), in many variant essential oils, resins and balsams, cinnamon oil, Peru balsam and Tolu balsam etc. These compounds are very important in almost biosynthetic ways.^[2-3] Cinnamic acid and its derivatives have a vital role in the synthesis of other important compounds and as precursors for the synthesis of commercially important cinnamic esters in perfume, cosmetic and pharmaceutical industries^{[4][5]}. Cinnamic acid and its derivatives have diverse bioactivities. This compound is able to inhibit the growing process of several species of bacteria and fungi^[6]. In addition, this compound has antioxidant, hepatoprotective, anxiolytic, insect repellent, antidiabetic and anticholesterolemic activities^[2].

Cinnamic acid derivatives are very versatile and have been featured in a variety of drugs. Various pharmacological profiles shown by cinnamic acid derivatives have a function as anti-TB, antioxidant, antitumor, anti-inflammatory, antidiabetic, anticholesterol etc.^[2]. Several cinnamic acid esters have been proven as antitumor, anti-inflammatory and sunscreen^[7]. Conventionally, cinnamic acid derivatives are synthesized by combining aromatic aldehydes and active methylene compounds with organic/inorganic bases. Condensation of aldehydes and malonic acid in several ester derivatives is applied in the pharmaceutical field^[8-9]. Reported synthesis of cinnamic acid with thionyl chloride produces acid halide. This acid halide can be reacted with alcohol to form cinnamic esters (cinnamyl esters). Several cinnamic esters isolated from Dutch propolis, benzyl caffeine, phenethyl caffeine, and cinnamoyl caffeine, have potential as anti-proliferative agents on carcinoma 26L5 with

EC50 values 0.288, 0.176, and 0.114 g/mL, Respectively. Phenethylcaffeine (caffeic acid phenethyl ester, CAPE) have several biological activities, such as antioxidant, anti-inflammatory, and inhibits tumor growth^[7].

Cinnamic acid-derived compounds are found in almost all plants but quantity is small so it cannot be depend on results of collected from extraction method or isolation of plant parts only. The increasing of amount production from cinnamic acid derivative compounds can be done by chemical synthesis^[10]. One of the derivatives cinnamate that can be synthesized is n-octyl cinnamate. This compound can be synthesized by esterification reaction between cinnamic acid and octanol with a concentrated sulfuric acid catalyst. Esterification is one of simple methods to change organic acids, in this case carboxylic acids to be ester derivative compounds. Esterification with catalyst acid help is common thing in making esters^[10]. This compound is a derivative from cinnamic acid at ester form with a saturated alcohol of n-octanol.

Several methods used to synthesize cinnamate derivatives, including reflux [11], microwave irradiation^[12]. The synthesis using this method needs a long time reaction and high temperature and requires high pressure. A long time, high temperature and pressure are the weaknesses of the conventional method, so it is needed for alternative method at synthesis cinnamate and its derivatives that can be resolve of conventional method, it is called sonochemical method (assisted of ultrasonic waves)^[13].

The using of sonochemical methods has become an alternative of green chemistry in this century. It is because this method is easy to do, efficient, with high yields, short time and environmentally friendly^[5-14-15], and also can be prevent the use of volatile things and toxic solvents by using harmless chemicals^[15].

This research is done by n-octyl cinnamate, supported by ultrasonic wave that know this activity as an antioxidant and anti-inflammatory *in vitro*. The compounds of synthesized result give elucidate on its structure with Infrared (IR), ¹H-NMR and ¹³C-NMR spectrophotometer.

II. MATERIALS AND METHODS

2.1 Chemicals

The materials used in this research include cinnamic acid (pa.), sulfuric acid (pa, Merck), n-octanol (pa, Merck), DPPH (pa, Sigma-Aldrich), ether (pa, Merck), ethyl acetate (pa, Merck), n-hexane (pa, Merck), methanol (pa, Merck), ether (pa, Merck), ethanol (pa, Merck), chloroform (pa, Merck), anhydrous NaHCO₃ (pa, Merck), Anhydrous MgSO₄ (pa, Merck), Bovine serum albumin (BSA), Tris buthyl saline (TBS), aquadest

2.3 Instrumentation

The reactor for the synthesis used the Bransonic Ultrasonic Bath series CPX1800H 40 KHz. Evaporator, melting point apparatus. The Infrared spectrum was measured using ATR-FTIR using carry 630 Agilent Technology at Semarang Pharmaceutical College. The melting point was measured using an electrothermal melting point. The ¹H-NMR, ¹³C-NMR, tests were carried out at Bandung Institute Technology with the instrument specs, namely ¹H-NMR (Agilent Technology; 500MHz); ¹³C-NMR (Agilent Technology; 125MHz).

2.4 Procedure Octyl Cinnamate Characterization

Synthesis of the compound n-octyl cinnamate using a procedure based on research by Nurul Hidajati et al (2008)[11] with several modifications. A total of ± 0.246 mol of cinnamic acid and 2.5 mol of n-octanol and 2.7 ml of concentrated H₂SO₄ were put into an erlenmeyer with boiling stone added. The all things was sonicated at 60°C for 5, 6 and 7 hours. The residue result poured into a separating funnel and give anhydrous NaHCO₃ till the atmosphere was neutral (pH=7). Furthermore, after the condition of the mixture has been neutral, then added ether. The ether phase was separated by adding MgSO₄ into a separating funnel and then homogenized for 5 minutes, then allowed for 20 minutes. Then separate with filter the mixture and accommodate in a porcelain cup, then evaporated filtrate got by using a water bath to remove n-octanol remain. Cooled filtrate then dried in oven at 60°C till get n-octyl cinnamate crystals.

2.5 Octyl Cinnamate Characterization

The precipitate obtained was tested for melting point using a melting point apparatus. The synthesized structures were characterized using FT-IR, ¹H-NMR, and ¹³C-NMR

2.6 Antioxidant test by in Vitro activities

The Determination of compounds antioxidant of synthesized with DPPH method accordance used by Ahmed, et al. (2013) with slight modifications. The synthesized compound was dissolved in ethanol to get 1000 ppm concentration. From these solution made a series of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm. Each of solutions series was on pipetted 2.0 mL and added 1 mL of 0.4 mM DPPH solution. The mixture was incubated for 30 minutes in a dark room and measure its absorbance at 515 nm wavelength. The blank is measured at the same wavelength as a sample by omitting the sample from the measurement. The positive control used was a

routine standard solution with 2-10 ppm concentration. The absorbance obtained from the measurement analyzed of antioxidant percentage activity.

With first linier.

$$\%inhibisi = \frac{A_{blanko} - A_{sampel}}{A_{blanko}} \times 100\%$$

2.7 Anti-Inflammatory test by In-vitro Activities

In vitro anti-inflammatory test based on the research method by (Williams et al., 2008). Stages testing activity of synthesis results on denaturation of Bovine Serum Albumin (BSA) A total of 5 ml of positive control solution consisted of 4.950 l BSA and 50 l diclofenac sodium solution. The control solution was made in various concentrations, namely 100 ppm, 10 ppm, 1 ppm, and 0.1 ppm. Each of the solution was vortexed, then incubated for 30 minutes at room temperature (27°C). After that, it was heated for 5 minutes at 72°C. Then it was left at room temperature (27°C) for 25 minutes. Then the turbidity was measured using a UV-Vis spectrophotometer at a wavelength of 660 nm. The percentage of inhibition of BSA denaturation can be count with this formula.

$$\% \text{ inhibisi} = \frac{\text{Abs kontrol negatif} - \text{Abs sampel}}{\text{Abs kontrol negatif}} \times 100\%$$

III. RESULTS AND DISCUSSION

The synthesis compound of n-octyl cinnamate in this study was obtained through an esterification reaction between cinnamic acid (0.246 mol) with octanol (2.5 mol) and 2.7 ml of concentrated H₂SO₄ through ultrasonic wave-assisted sonochemical methods[14][8]. The Reaction can be seen in figure 1.

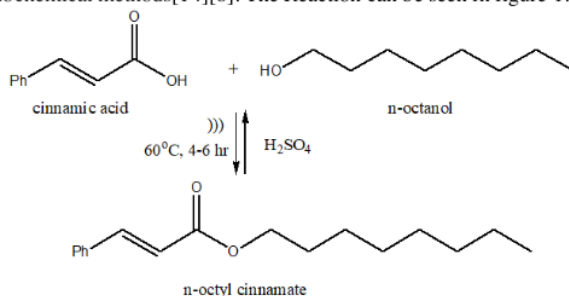


Figure 1. Esterification reaction on cinnamic acid with n-octanol

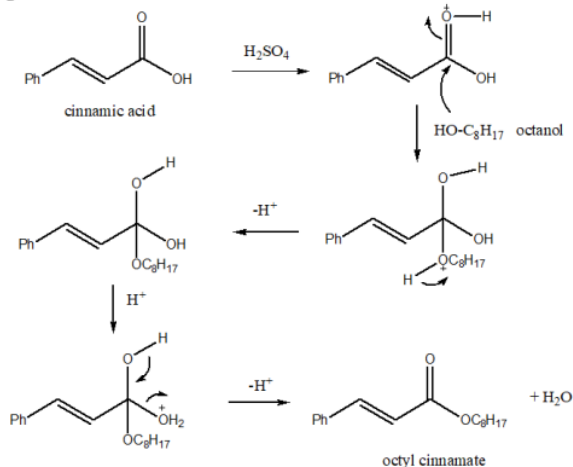


Figure 2. Mechanism of esterification cinnamic acids and octanol

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Table 1. Results of the reaction between cinnamic acid and n-octanol

No	Time (hours)	Temperature(⁰ C)	Catalyst	Product Weight (g)	% Yield
1.	5	60	H ₂ SO ₄	0,1393	23,02%
2.	6	60	H ₂ SO ₄	0,1671	27,61%
3.	7	60	H ₂ SO ₄	0,2371	39,18%

Based on table 1 above, it can be seen that the largest percent yield is produced at 7 hours. Temperature conditions of 60⁰C with sonication 7 hours time call the optimum conditions for these reactions. The esterification process between cinnamic acid and primary alcohol, namely n-octanol, produces n-octyl cinnamate cinnamic esters. The use of primary alcohols in this synthesis can be attributed to their reactivities. Primary alcohols have a smaller steric hindrance than secondary and tertiary alcohols, with a smaller steric hindrance. So, primary alcohols easier to get effectively interaction with cinnamic acid than secondary and tertiary alcohols^[10].

The synthesis of these compounds is carried out with ultrasonic waves help in a sonicator. Ultrasonic irradiation would increase the reaction between cinnamic acid and n-octanol through cavitation mechanism^[16]. The results of the organoleptic test showed that the synthesized compound was a white powder, odorless and had a melting point on 242.1⁰C-244.2⁰C.

3.1 Octyl Cinnamate Characterization

The results test of structural elucidation by using FT IR spectrophotometry can be seen in Figure 3.

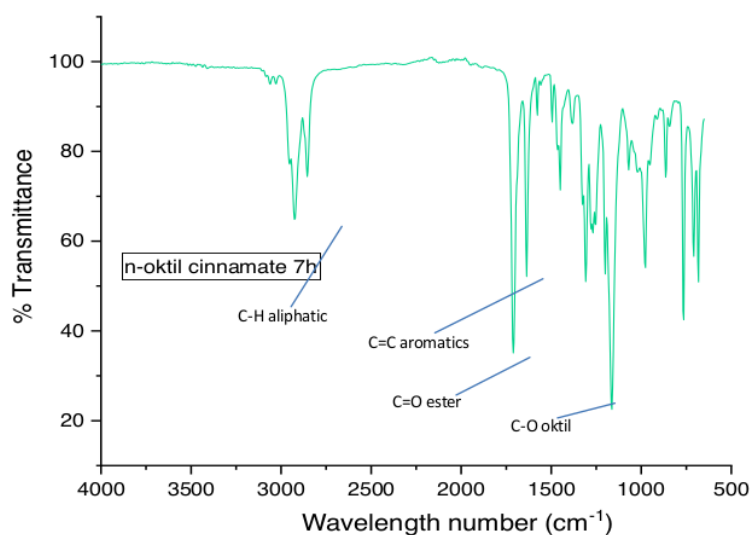


Figure 3. Spectrum FTIR from n-octyl cinnamate

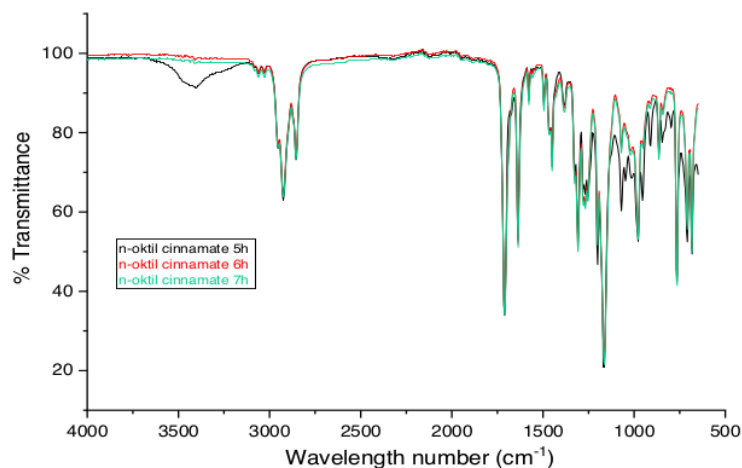


Figure 4. Overlays FTIR n-octyl cinnamate 5,6,dan 7 hours

Based on the spectrum overlay (figure 4) between the synthesized product with sonication 5, 6 and 7 hours time, it can be seen that the synthesis results show a slightly different spectrum profile between third of them. The results of the 5-hour FTIR spectrum showed that there were still OH groups from the alcohol used, namely octanol that was indicated by a widening vibration, while the 6 and 7-hour FTIR spectrum showed similarities in the specific functional groups. This explanation showed that at 6 hours of sonication, the OH group of alcohol did not appear.

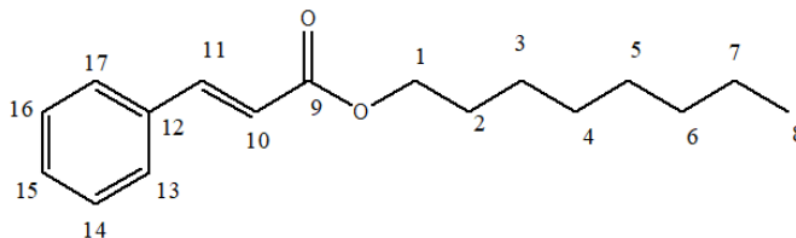


Figure 5. Structure n-octyl cinnamate

Table 2. Data of $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ n-Octyl Cinnamate

C position	δC (ppm)	δH (ppm) (ΣH , multiplisitas, J (Hz))
1	64,9208	4,2016 (2H, t)
2	28,8835	1,7050 (2H, m)
3	26,1370	1,2987 (2H, m)
4	29,4040	1,2987 (2H, m)
5	29,3497	1,2987 (2H, m)
6	31,9477	1,2987 (2H, m)
7	22,7990	1,3117 (2H, m)
8	14,2417	0,8869 (3H, t)
9	167,2920	-
10	118,4632	6,4622 (1H, d, 16)
11	147,1275	7,7933 (1H, d, 16)
12	144,7157	-
13, 17	128,2037	7,685 (2H, dd, 16)
14,16	129,1180	7,5642 (2H, m)
15	129,0194	7,5362 (1H, m)

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The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ results of synthesized compounds showed a suitable chemical shift for n-octyl cinnamate (Table 1). The spectra of $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ were measured using CDCl_3 solvent with 500 MHz frequency. The results of the $^1\text{H-NMR}$ spectrum showed some absorption in 7,0-8,0 ppm area. The chemical shift that appears in several peaks indicating several atomic positions that correspond to n-octyl cinnamate structure. Chemical shift in 7,5642-7,685 ppm area indicates aromatic compounds. Octyl group on chemical shift 0,88-4,2016 ppm. C=C at a chemical shift of 7,7933 ppm. The $^{13}\text{C-NMR}$ spectrum showed a chemical shift indicating the number of C atoms was 17 for n-octyl cinnamate. The C=O ester group was shown at a chemical shift of 167,290 ppm, the aromatic group was shown at a chemical shift of 128,2037-144,7157 ppm. The octyl C group was shown at a chemical shift of 14,2417-28,8835 ppm. C-O group on a chemical shift of 64,9208 ppm. Based on the interpretation data from FTIR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ shown the result of the synthesis was n-octyl cinnamate.

3.2 Antioxidant Activity In Vitro

The testing of antioxidant activity using the DPPH method is a good method to measure antioxidant efficacy in vitro^[17]. It was done to evaluate the antioxidant activity of the synthesized compounds^[18]. The principle of antioxidant activity test method is quantitative with measuring the DPPH radical capture by one of compound that has antioxidant activity using UV-Vis spectrophotometry so, the value of free radical activity will be known with C50 values (Inhibitory). concentration^[19]. The DPPH radical antidote activity of synthesized compound compared with ascorbic acid at same concentrate, the result of antioxidant measure expressed as % DPPH radical inhibition. The radical reduction of DPPH determined by taking its absorption at 515.4 nm wavelength. The following table was the result of measuring the antioxidant activity of synthesize compounds spectrophotometri

Table 3. Antioxidant Activity n-Octyl Cinnamate

Concentration $\mu\text{g/mL}$	Absorbance	% Inhibition	Equality $y = bx+a$	EC50 $\mu\text{g/mL}$
Blanko	0,755	0		
10	0,499	33,91		
20	0,456	39,60	$y = 0,6092x + 28,85$	34,71
30	0,369	51,13	$R^2 = 0,9412$	
40	0,350	53,64		
50	0,322	57,35		

Based on the results of table 3 above, the EC50 value of the synthesized n-octyl cinnamate compound obtained from the calculation of the linear equation 34.71 g/mL, that can be assumed the antioxidant activity of synthesized compound was strong. It can be proven by increasing the concentration of synthesized compound, so, % inhibition also increases. It can be seen from the curve of relationship between concentration and % inhibition below.

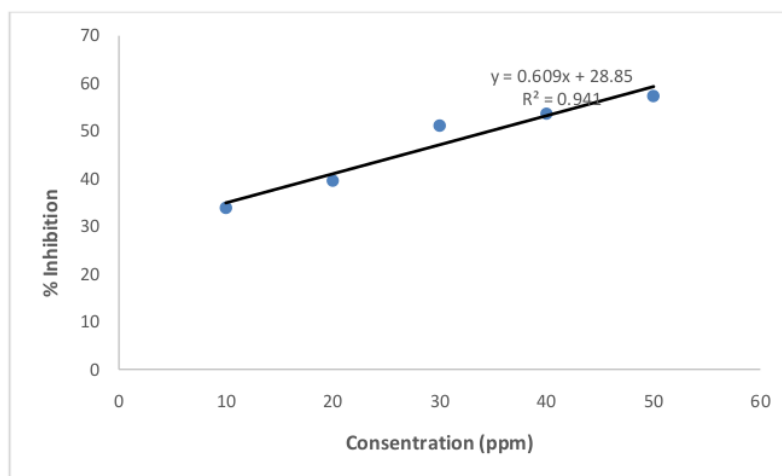


Figure 6. Linear Regression Curve for n-octyl cinnamate

The positive control used was vitamin C. The positive control used in this antioxidant activity test was to determine how strong the antioxidant potential of synthesized compound when compared to vitamin C. Based on the calculation of the EC50 value of vitamin C was 7.93 g/mL. So it could be assumed that compound n-octyl cinnamate has a strong antioxidant activity close to positive control. The synthesized compound has strong antioxidant activity because derivative of cinnamic acid was more active than its precursor compound, it is because of addition of octyl group with a longer chain causes allosteric properties faster than its precursor, so, increase its antioxidant activity^[18]. The compound n-octyl cinnamate was soluble in organic solvents while insoluble in water can be assumed that compound has some lipophilic characteristics, it causes the antioxidant activity in this compound better than its precursor^[18-17].

3.3 Anti-Inflammatory Activity In-vitro

In this study, in vitro anti-inflammatory activity was tested on synthesized compound using Bovine Serum Albumin (BSA) method, by observing the effect of protein denaturation inhibition. The measurement of BSA were performed to eliminate live specimens in the drug development process^[20-21]. Based on William, et al (2008) explained compounds that have protein denaturation inhibition percentage >20% were compounds that have anti-inflammatory activity^[22]. Diclofenac sodium used as a positive control using BSA. In this method, concentration range from 0.1 ppm to 100 ppm was used.

Diclofenac sodium as a positive control had denaturation protein inhibitory activity at 100 concentration of ppm with % of inhibition was 57.69%. While synthesized compound, namely octyl cinnamate at 100 concentration of ppm, got % inhibition was 55.56%, the results showed that the inhibitory effect of protein denaturation increased with increasing concentration of compound octyl cinnamate.

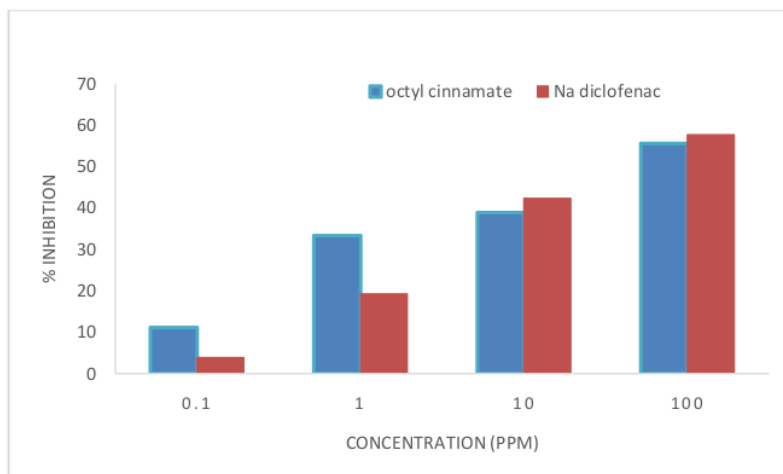


Figure 6. curve % inhibition inhibisidiflofenac sodium dan octil cinnamate

Based on the curve above, showed that the % inhibition of the octyl cinnamate compound was close to the % inhibition in positive control, namely sodium diclofenac. It was because of octyl cinnamate compound has an ester group (COO-R) that causes an increase in anti-inflammatory activity^[22-23].

IV. CONCLUSION

Based on the results of the study, it can be concluded that compound of n-octyl cinnamate can be synthesized using an esterification reaction between cinnamic acid and octanol with sonochemical sulfuric acid catalyst. The 7 hours showed the optimum time for the synthesis of octyl cinnamate with a yield of 39.18%. Sonochemistry gives a good results in short reaction time for synthesis of octyl cinnamate. This compound has a potential of antioxidant and anti-inflammatory in vitro.

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