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SYNTHESIS OF *n*-OCTYL CINNAMATE USING DCC COUPLING REAGENT ASSISTED BY ULTRASONIC WAVE AND ANTIOXIDANT ACTIVITY TESTS

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ABSTRACT

n-Octyl cinnamate is a cinnamic acid derivative that can be synthesized by an esterification reaction between cinnamic acid and n-octanol with the addition of a DCC coupling reagent (N,N'-dicyclohexylcarbodiimide), which is varied into three concentrations equivalent to 1:0.5, 1:0.75, and 1:1 moles of cinnamic acid. Ultrasonic waves assisted in the synthesis of n-octyl cinnamate at 2-8°C for 4 hours. The obtained n-octyl cinnamate was characterized by its organoleptic properties, solubility, melting point, TLC, FTIR-ATR, GC-MS, and antioxidant activity was measured using the DPPH assay. In the results, n-octyl cinnamate was obtained using DCC coupling reagent in 11.85; 14.83; and 35.83 % yields, respectively. This ester dissolves in methanol, ethanol, chloroform, ether, and n-hexane but is insoluble in water. It had a melting point range of 151.4-157.8 ºC. TLC shows that this ester has a different Rf value from that of the reactant. The FTIR spectrum showed suitable bonds with n-octyl cinnamate, as can be seen from its aromatic bands (C=C and C-H), which are characterized by the stretching of C=O and C-O bonds, aliphatic C=C, and aliphatic C-H absorptions. The results from the GC-MS analysis of the targeted compound appeared at 17.184 minutes with a 44.74% purity rate using synthesized n-ocytl cinnamate with the addition of 0,75 equivalent of DCC. The antioxidant activity test using the DPPH assay showed an IC50 value of 93.3375 ppm, indicating strong antioxidant activity.

Keywords : *antioxidant, esterification, ultrasonic wave, N,N'-dicyclohexylcarbodiimide, n-octyl cinnamate*

INTRODUCTION

Cinnamate ester is a derivative of cinnamic acid with higher lipophilicity, which enables it to cross lipid membranes more effectively and provide greater protective effects against degenerative diseases than cinnamic acid . According to , n-octyl cinnamate was synthesized by reacting cinnamic acid with n-octanol using a sulfuric acid catalyst and ultrasonic wave assistance. The synthesis process required 7 hours at 60 °C. Sulfuric acid is a commonly used catalyst in Fischer esterification, which accelerates carboxylic acid activation and the reaction rate. However, this method often yields low .

Activators or coupling reagents are often used in esterification syntheses. The DCC (N,N'-dicyclohexylcarbodiimide) coupling reagent was chosen for its reactivity in bridging the two compounds to facilitate the reaction. This process can occur at room temperature or lower,

resulting in an increased yield. The concentration of DCC added during esterification affects the rate of carboxylic acid group activation, influencing the yield of the synthesized compound . According to , the coupling reagent can be added at a ratio of 1:1.5, relative to the moles of the reactant compound used.

The synthesis of n-octyl cinnamate via Steglich esterification using a DCC coupling reagent with varying ratios of 1:0.5, 1:0.75, and 1:1 relative to moles of cinnamic acid was performed to obtain n-octyl cinnamate, which was tested as an antioxidant agent to counteract free radicals. The esterification reaction was assisted by ultrasonic waves from the sonication process, allowing for shorter reaction times and easier execution than conventional methods .

Free radicals are reactive molecules that attack the surrounding molecules and cause oxidative stress in the body. Therefore, antioxidants are required to inhibit oxidation . N-Octyl cinnamate is a compound containing a C=C bond with potential antioxidant properties. Antioxidant strength can be measured using various methods including the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. DPPH is a radical compound that can be neutralized by receiving electrons from a compound. This method is specific, simple, and widely used for testing antioxidant compounds, requiring readily available reagents and no specialized instruments **.**

n-Octyl cinnamate was synthesized by reacting cinnamic acid with n-octanol using a DCC coupling reagent assisted by ultrasonic waves. The resulting n-octyl cinnamate was characterized by organoleptic tests, solubility, melting point, Thin Layer Chromatography (TLC), Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR), and Gas Chromatography-Mass Spectrometry (GC-MS). The antioxidant activity of the synthesized n-octyl cinnamate was also evaluated using the DPPH assay, expressed as the IC_{50} value.

RESEARCH METHODS

Equipment

Volumetric pipette, dropping pipette, beaker glass, Erlenmeyer flask, tripod, clamp, porcelain dish, separatory funnel, oven, filter paper, chamber, waterbath, Sonicator (Branson 1800), FTIR (*Agilent Cary 630*), TLC plates (*Silica Gel 60 GF_{254}*), melting point apparatus *(Electrothermal IA9100),* GC-MS (*Shimadzu QP 2010 SE*), UV-Vis Spectrophotometer (*Shimadzu UV-1700 PharmaSpec*).

Materials

Cinnamic acid, n-octanol, anhydrous magnesium sulfate *(Merck, technical grade)*, anhydrous sodium bicarbonate, n-hexane, ethyl acetate, methanol, DPPH reagent, and vitamin C standard *(Merck, pro analyze).*

Research Procedure

1. Synthesis of n-Octyl Cinnamate

n-Octyl cinnamate was synthesized according to the method described by , with slight modifications. Approximately 0.246 mol of cinnamic acid and 2.5 mol of n-octanol were added to an Erlenmeyer flask, followed by the addition of DCC (N,N'-dicyclohexylcarbodiimide) coupling reagent in varying mole ratios to cinnamic acid, namely 1:0.5, 1:0.75, and 1:1. The obtained mixture was sonicated at cold temperature (2-8°C) for 4 hours. The sonication process was continued with pH neutralization by adding saturated $NaHCO_3$ solution until a pH of 7 was reached. A separatory funnel was used to extract the mixture, which formed two layers. The upper layer, consisting of the organic phase, was collected in a porcelain dish and anhydrous MgSO4 was added. The mixture was allowed to stand for 20 minutes. MgSO4 powder was separated from the mixture using filter paper, and the filtrate was collected in a porcelain dish. The filtered mixture was then heated in a water bath at 100°C until the odor disappeared, and subsequently dried in an oven at 60-70°C until a white powder of n-octyl cinnamate was formed.

2. Characterization and Identification of Synthesized Compounds

a. Solubility Test

Several test tubes were prepared and filled with various solvents including water, methanol, ethanol, chloroform, ether, and n-hexane. A small amount (around 20 mg) of the synthesized compound was added to each tube, and its solubility was observed. The test is soluble if all the added synthesized compounds perfectly dissolve in the solvent and create a clear solution.

b. Melting Point Test

The synthesized compound was placed in a capillary tube and tested using a melting point apparatus at a temperature increase of 1°C/min. The temperature range in which the compound melted was recorded.

c. Thin Layer Chromatography (TLC) Test

The eluent, which contained n-hexane, ethyl acetate, and methanol (80:15:5), was saturated in a chamber with saturation paper. Samples of the synthesized and reference compounds (cinnamic acid and n-octanol) were spotted on a TLC plate (Silica GF_{254}). The plate was eluted with a saturated eluent and observed under UV light at 254 nm and 366 nm.

d. Structural Identification using FTIR-ATR

An FTIR instrument (Agilent Cary 630) was used to measure the empty sample compartment (air) and set to the background spectra of the FTIR spectrophotometer before testing the synthesized compound and reference compounds (cinnamic acid and n-octanol). The obtained spectra showed the functional groups present in the compounds.

e. Structural Identification using GC-MS

The synthesized compound was dissolved in methanol (10 mg in 5 mL) and injected into a GC-MS instrument (Shimadzu QP 2010 SE) using an autoinjector syringe. The initial temperature of 80°C was increased to 300°C at a rate of 10°C/min and the flow rate was set at 0.46 ml/min. Chromatograms and mass spectrum of the compounds were obtained.

3. Antioxidant Activity Test using DPPH Assay

The antioxidant activities of the synthesized compounds were measured using the DPPH assay. A test solution was prepared by dissolving 50 mg of n-octyl cinnamate crystals in 50 mL of methanol (1000 ppm). A series of sample concentrations (20, 40, 60, 80, and 100 ppm) were prepared in 10.0 mL volumetric flasks. Each concentration was then pipetted (0.2 mL) using a micropipette and added to 5 mL DPPH solution. The mixture was then incubated for a specified Operating Time (OT) in the dark. The absorbance of the incubated solution was measured. The antioxidant activity of the samples was determined by calculating the IC_{50} value, which is the concentration required for 50% inhibition of DPPH.

Data Analysis

1. Calculation of % Yield of n-Octyl Cinnamate

The synthesized n-octyl cinnamate crystals were weighed to determine the % yield by comparing the actual weight of the synthesized compound with the theoretical weight. The calculation of the percentage yield obtained is

$$\text{Yield (\%)} = \frac{\text{Weight of synthesized compounds } \backslash\backslash(g\backslash\backslash)}{\text{Theoretical weight of compound } \backslash\backslash(g\backslash\backslash)} \times 100\ \% \qquad (1)$$

2. Analysis of Antioxidant Activity of n-Octyl Cinnamate through DPPH (1,1-diphenyl-2-picrylhydrazyl) Scavenging Percentage

The absorbance of the n-octyl cinnamate crystals obtained was then calculated as a percentage of antioxidant activity. Antioxidant activity was calculated as a percentage of inhibition relative to the control using the following equation:

$$\% \text{ Antioxidant Activity} = \frac{SampleAbsorbance}{\text{Control Absorbance}} \text{ x } 100\ \% \quad (2)$$

The percentage of antioxidant activity using the DPPH assay was calculated to determine the IC50 value using the linear regression equation of the synthesized compound concentration versus inhibition percentage. The IC50 value was calculated and the category was determined according to Table I.

Table I. Category of Antioxidant Activity Strength In Vitro Against DPPH

Intensity of IC_{50}	Consentration
Very Strong	<50 ppm
Strong	50-100 ppm
Moderate	101-150 ppm
Weak	151-200 ppm

Source : Surjanto et al., 2019.

RESULTS AND DISCUSSION

The synthesis of n-octyl cinnamate was carried out through Steglich esterification by reacting cinnamic acid (1.0934 grams, 0.246 mol) with n-octanol (11.75 mL, 2.5 mol) using various ratios of DCC (1:0.5, 1:0.75, and 1:1) relative to the moles of cinnamic acid. The esterification reaction begins with the deprotonation of the -OH group of cinnamic acid by the nitrogen atom of DCC, forming a carboxylate ion (-RCOO-) with nucleophilic properties. DCC undergoes protonation, which increases its electrophilic properties. The carboxylate ion of cinnamic acid then attacks the imide bond of DCC, forming an intermediate compound called O-acylisourea. This compound has an electron-deficient and highly reactive central carbon atom (-C-).

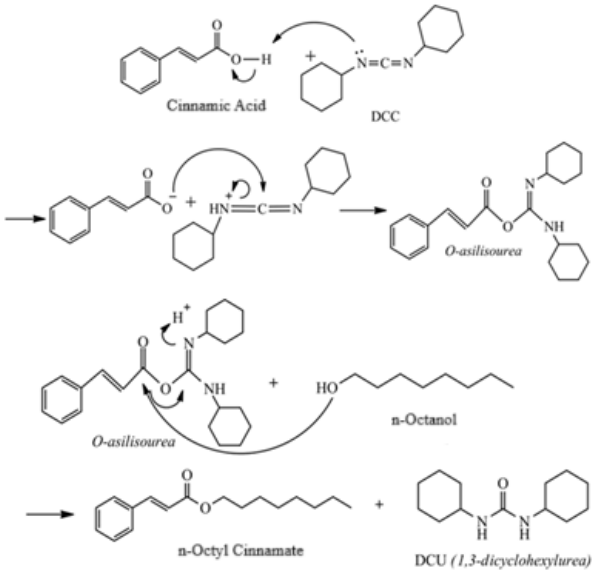

Figure 1. Mechanism of Synthesis of n-Octyl Cinnamate through Steglich Esterification

The isourea group (-N=C=N-) of DCC is highly reactive and susceptible to nucleophilic attack. Figure 1 shows that the oxygen atom of acylisourea is electronegative, pulling electrons from the carbonyl bond (-C=O-), resulting in a partial positive charge on the carbon atom. This highly reactive compound then reacts with n-octanol, which has a nucleophilic -OH group that attacks the carbonyl bond. The resulting compound then releases a proton and delocalization

occurs, resulting in the release of oxygen from the DCC as dicyclohexylurea, a byproduct, and the formation of the target compound, n-octyl cinnamate .

The synthesized compound was tested organoleptically, including its color, shape, and odor, which appeared as a white powder with no distinct odor. This result is consistent with the findings of a previous study by Table II.

Table II. % Yield of Synthesized Compound

Replication	Ratio of Cinnamic Acid to DCC		
	1:0,5	**1:0,75**	**1:1**
1	16,42 %	16,58 %	30,84 %
2	10,72 %	19,92 %	36,60 %
3	8,42 %	9 %	41,04 %
Total	35,56 %	44,50 %	107,48 %
Average	11,85 %	14,83 %	35,83 %

Table II shows that the compounds synthesized at ratios of 1:0.5, 1:0.75, and 1:1 had average % yields of 11.85%, 14.83%, and 35.83%, respectively. This indicates that the yield increased as the concentration of the coupling reagent DCC increased. The addition of DCC facilitated the reaction between the reactant compounds by increasing the number of reactive carbonyl forms that reacted with n-octanol, resulting in a higher product yield in a shorter time. Solubility testing showed that the synthesized compound was insoluble in water, but soluble in methanol, ethanol, chloroform, ether, and n-hexane. This indicates the polarity of the compound in various solvents, and the synthesized compound has semi-polar to non-polar properties and can be quickly dissolved in methanol or ethanol.

Table III. Results of Melting Point Test

Replication	Melting Point (°C)		
	1:0,5	**1:0,75**	**1:1**
1	145,5 - 150,7°C	151,7 - 158,2°C	139,3 - 161,8°C
2	144,2 -150,4°C	152,4 -159,4°C	134,3 - 158,4°C
3	144,7 -150,3°C	151,4 -157,8°C	140,4 - 166,3°C
Melting Range	5,2 - 6,2°C	6,4 - 7°C	22,5 - 24,6° C

The result of the melting point examination of a compound is said to be pure if it has a melting point range of 1-2°C. The test results in Table III show that the melting point of the synthesized compound is not yet pure because it has a broad melting point range.

The results of the melting point test showed that the synthesized compound was not yet pure, as it had a wide melting point range. Further testing using thin-layer chromatography (TLC) was conducted to determine the elution pattern, which formed three spots. The first spot had an Rf value of 0.1-0.25, similar to that of cinnamic acid, indicating that the synthesized compound still contained unreacted cinnamic acid. The second spot, with an Rf value of 0.4-0.5, was suspected to be a byproduct of the esterification reaction using DCC, namely DCU. The third spot, with an Rf value of 0.85-0.9, was suspected to be the target compound, n-octyl cinnamate, because of its longer molecular structure and nonpolar nature compared to cinnamic acid.

The FTIR spectrum of the synthesized compound showed the disappearance of the -OH group, indicated by a broad absorption peak at 3700-3230 cm^{-1}, the disappearance of the specific C-O bond of cinnamic acid at 1315-1200 cm^{-1}, and the formation of a C-O ester bond (Dachriyanus, 2004).

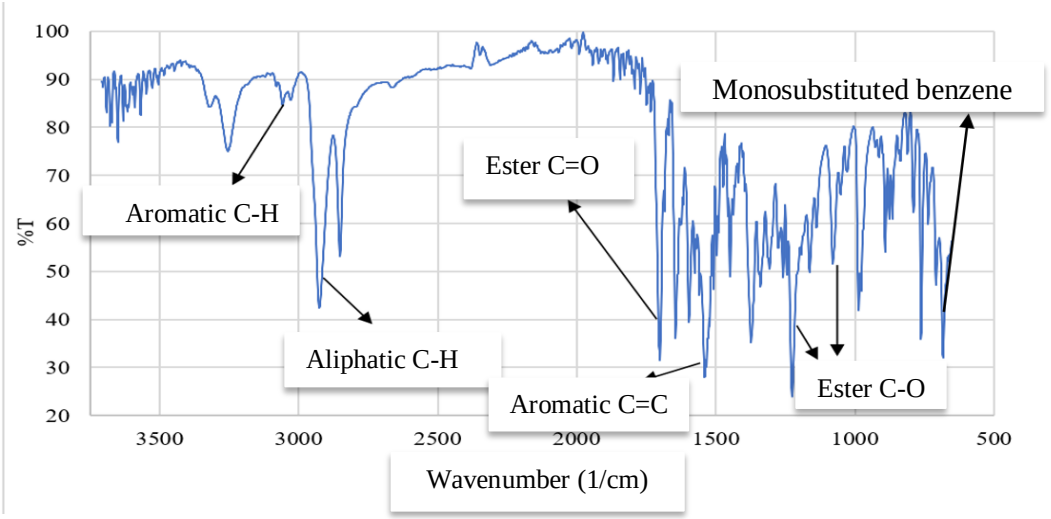

Figure 2. FTIR Spectrum of n-Octyl Cinnamate

The spectrum of the synthesized compound in Figure 2 shows an absorption at 682 cm^{-1}, which is the absorption of a monosubstituted benzene ring. The benzene ring, which has aromatic C=C bonds, absorbs 1675-1500 cm^{-1}, and aromatic C-H bonds between 3150-3000 cm^{-1}. The FTIR spectrum showed that the aromatic C=C bond appeared at an absorption of 1541 cm^{-1}, whereas the aromatic C-H bond appeared at 3058 cm^{-1}. The n-octyl cinnamate compound had an aliphatic C=C bond with an absorption value of 986 cm^{-1}. The spectrum shows C=O absorption at 1702 cm^{-1}. The C-O bond in an ester compound with an aromatic ring has two absorptions. The first absorption in the spectrum was read at 1226 cm^{-1}, whereas the second absorption for the ester derived from a primary alcohol appeared in the spectrum at 1073 cm^{-1}. An organic compound essentially has a C-H bond, and the n-octyl cinnamate compound has an aliphatic C-H bond that appears at 2926 cm^{-1} within the range of 3000-2700 cm^{-1}. The spectrum shows the presence of $-CH_3$ and $-CH_2-$ bonds, which are the methyl and methylene groups, respectively. The $-CH_3$ bond appears in the spectrum at 1396 cm^{-1}, which falls within the absorption range of 1440-1388 cm^{-1}, whereas the $-CH_2-$ bond typically appears at an absorption near 1420 cm^{-1}. The spectrum shows that this bond has an absorption of 1420 cm^{-1} (Colthup et al., 1990; Dachriyanus, 2004).

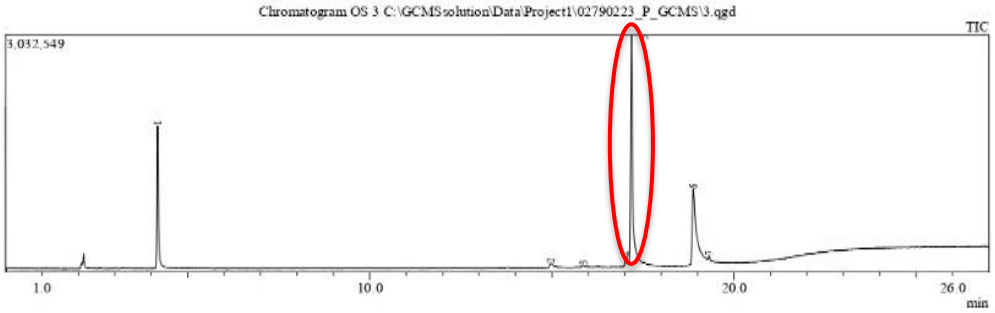

Figure 3. Chromatogram of Synthesized Compound

The GC chromatogram shows that the formed n-octyl cinnamate compound is still not pure, as indicated by the formation of 7 peaks that can be seen in Figure 3. The peak of n-octyl cinnamate was separated from other peaks, namely peak 5 with a retention time of 17.184 minutes and an abundance of 44.74%.

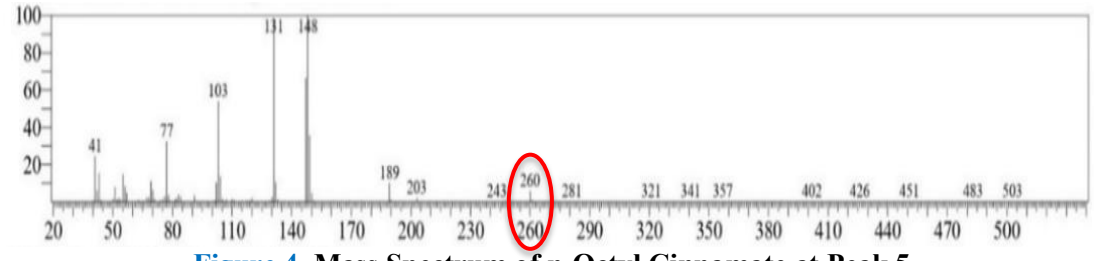

Figure 4. Mass Spectrum of n-Octyl Cinnamate at Peak 5

Analysis of the mass spectrum shown in Figure 4 revealed the first peak as the molecular ion at 260 m/z, which is the molecular weight of the target compound n-octyl cinnamate with the molecular formula $C_{17}H_{24}O_2$. Fragmentation of the compound produced peaks at 260, 203, 189, 148, 131, 103, 77, and 41 m/z, with a possible fragmentation pattern of n-octyl cinnamate as follows.

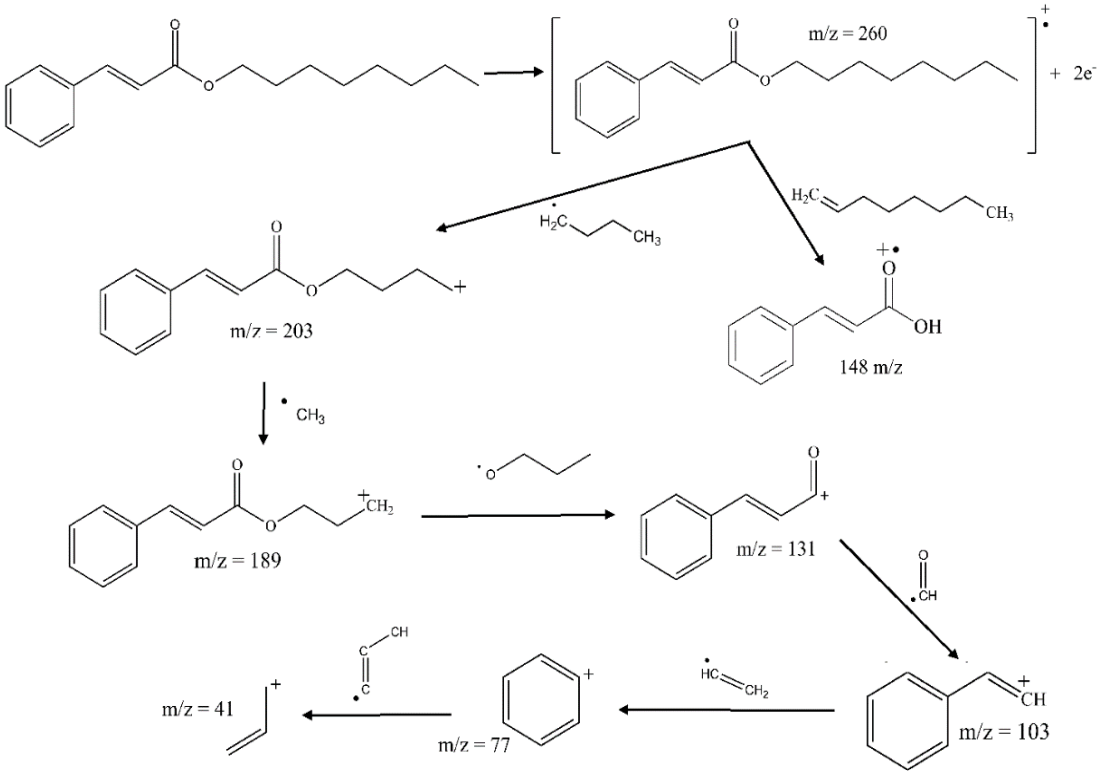

Figure 5. Fragmentation Pattern of n-Octyl Cinnamate

Figure 5 illustrates the fragmentation pattern, where the second pattern appeared at 203 m/z, representing the compound ion after releasing a butyl radical (C_4H_9)$^{\cdot}$. The ion then formed a third fragmentation pattern with a peak at 189 m/z after the release of a methylene radical ($CH_2^{\cdot}$). The fourth fragmentation pattern appeared, with the highest peak at 148 m/z, releasing an octene (C8H16). The fifth pattern appeared at 131 m/z, releasing a propoxy radical (C_3H_6O)$^{\cdot}$. The sixth fragmentation pattern showed a peak at 103 m/z after the release of a formyl radical (CHO)$^{\cdot}$, followed by the seventh fragmentation pattern with a peak at 77 m/z after releasing a vinyl radical ($C2H3^{\cdot}$) and eighth pattern with the release of $C_3H^{\cdot}$ radical.

The synthesized compound was subjected to in vitro antioxidant activity measurements using a DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Antioxidant measurement was continued by preparing a series of concentrations of vitamin C standard solution and n-octyl cinnamate samples. The vitamin C standard was prepared with a series of 10, 15, 20, 25, and 30 ppm, whereas the n-octyl cinnamate sample solution was prepared with concentrations of 20, 40, 60, 80, and 100 ppm. The antioxidant strength was determined as the IC_{50} value. Based

on the above data, the IC_{50} values of the vitamin C standard and sample are presented in Table IV.

Table IV. Antioxidant Activity Test of Synthesized n-Octyl Cinnamate Compound

No.	Solution	Consentration (ppm)	% Inhibition	IC_{50} (ppm)
1	Vitamin C	10 15 20 25 30	12,65 19,80 35,27 42,41 53,23	28,7223 (Very Strong)
2	Syntesized n-Octyl Cinnamate	20 40 60 80 100	7,67 15,34 28,55 40,27 57,21	93,3375 (Strong)

The antioxidant strength of a compound was determined as the IC_{50} value, which is the concentration of the test solution that can neutralize 50% of the DPPH radical activity. The IC_{50} value is inversely proportional to the antioxidant strength of a compound, that is, the smaller the IC_{50} value obtained, the stronger the antioxidant potential Based on the results in Table IV, the average IC_{50} value of the antioxidant activity of the vitamin C standard was 28.7223 ppm, and that of the n-octyl cinnamate compound was 93.3375 ppm.

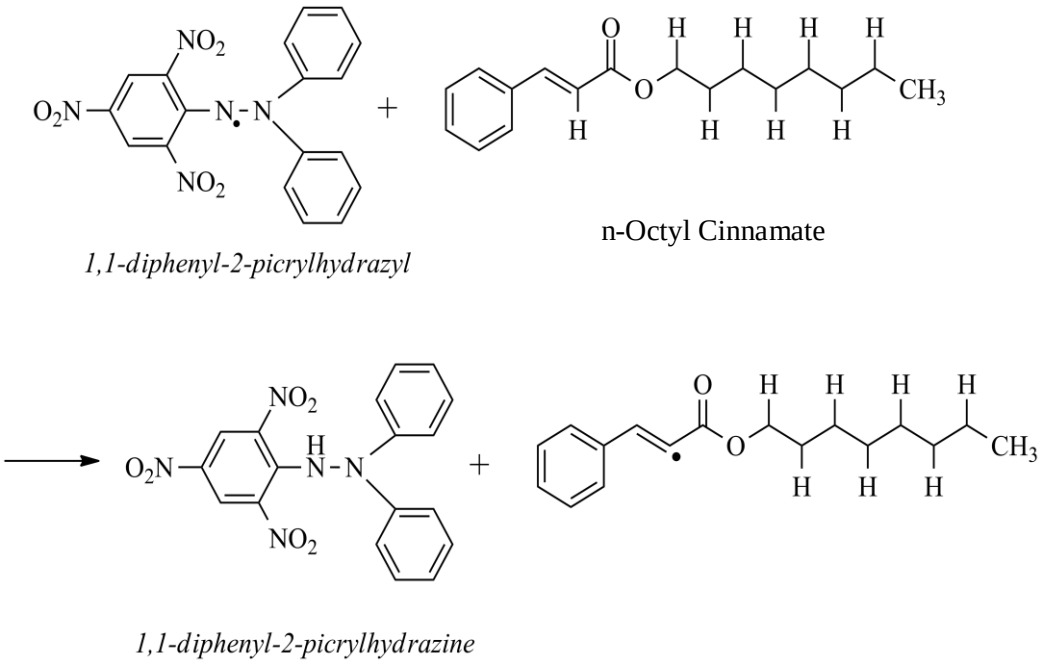

Figure 6. Reaction of n-Octyl Cinnamate Compound with DPPH Radical

Figure 6 shows the neutralization of DPPH radicals by hydrogen transfer based on redox reactions. The number of hydrogen atoms drawn can increase antioxidant strength . The donated hydrogen atom reduces the intensity of the purple color of the DPPH compound. The donation of hydrogen atoms to quench DPPH radicals starts from the C = C double bond near the carbonyl (-C=O-) group, which has one hydrogen atom and is close to the octyl chain. The long octyl chain in the compound increases its lipophilic properties, resulting in reduced steric

hindrance, and thus weaker intramolecular hydrogen bonds. Hydrogen atoms are easier to release and reach the radical center faster, resulting in a higher DPPH radical scavenging activity .

CONCLUSION

Based on the research results, it can be concluded that the synthesis of n-octyl cinnamate has the best yield percentage in the variation of DCC (N,N'-dicyclohexylcarbodiimide) concentration, with a reaction yield of 14.83% at a concentration of 1:0.75. The compound has an IC_{50} value of 93.3375 ppm and is categorized as a potent antioxidant.

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