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A GREEN SYNTHESIS OF ACETYL EUGENOL BY SONOCHEMICAL METHOD AND POTENTIAL AS ANTI-INFLAMMATORY IN-VITRO

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ABSTRACT

Clove oil is an essential oil from the clove plant (*Syzygium aromaticum*) containing eugenol compounds. One of the properties of eugenol is as an anti-inflammatory with a mechanism of inhibition of prostaglandin synthesis and neutrophil chemotaxis. Several derivatives of eugenol have active compounds that have been developed into new drug compounds as anti-inflammatory such as acetyl eugenol (4-allyl-2-methoxyphenyl acetate). This study aims to determine the % yield of acetyl eugenol produced from synthesis using ultrasonic 0.0323 mol of eugenol added to Erlenmeyer, and 0.25 mol of 10% sodium hydroxide was added. The mixture was put in a sonicator for 15 minutes and heated at 60^0C. Then, 0.0974 mol acetic anhydride was reacted with DCC, added to the mix and sonicated with time variations (60, 80, and 100 minutes). The chemical structure was elucidated using FTIR, ATR, and GC-MS. The synthesized % yield is 32.75%. Based on the interpretation data from FTIR, 3405 cm^{-1} is an O-H group (free phenol), 1405 cm^{-1} is an alkene group (C=C) aliphatic, and 1560 cm^{-1} is an aromatic compound with the presence of a C=C aromatic bond. The presence of the (C-O) ether group is indicated in the wave number at 1301 cm^{-1}. The C=O ester bond in the ester group is shown at 1700 cm^{-1}. GC-MS shows that the synthesized compound has a molecular ion with m/z = 206. According to the molecular weight of acetyl eugenol of 206 g/mol, it can be concluded that acetyl eugenol was successfully synthesized. The most stable ionic fragment, 37, has a molecular weight of m/z = 164. The activities of anti-inflammatory, acetyl eugenol compounds at 400 concentration ppm get % an inhibition of 32.20%.

Keywords: *Acetyl eugenol, Antioxidant, Decyclocarbodiimide, Sonochemical, Synthesis*

INTRODUCTION

Covid-2019 (Corona Virus Disease 2019) is caused by the SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) virus. The disease has become a global pandemic, with >12 million confirmed cases and more than half a million deaths in more than 200 countries [1,2]. Pathophysiologically, Covid-19 infection has an aggressive inflammatory response to damage to the respiratory tract, causing a reaction in the form of inflammation [1,3]. Inflammation from adipose tissue generates chronic and systemic metabolic alterations leading to dyslipidemia, hypertension, CVD and diabetes, thus increasing the risk of infection by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) [4]. It is thought that SARS-CoV-2, as

SARS-CoV and MERS-CoV, suppress anti-viral type IFN-g responses in the early stage of infection, leading to an un- controlled viral replication. This mechanism later leads to an influx of neutrophils and monocytes/macrophages, resulting in the hyper-production of pro-inflammatory cytokines that can damage lung tissue (i.e. pneumonia, acute respiratory distress syndrome) [5].

One of the clinical symptoms of inflammation (inflammation) is fever, which can generally be caused by an infection or as a result of tissue damage. Anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) have a biosynthetic mechanism of NSAIDs that can inhibit the formation of prostaglandins. The enzymatic activity involved in prostaglandin synthesis, where these drugs can inhibit the action of the cyclooxygenase (COX) enzyme [6,7].

Clove oil is an essential oil from the clove plant (*Syzygium aromaticum*) containing eugenol compounds [8]. One of the properties of eugenol is as an anti-inflammatory, with the mechanism inhibiting the synthesis of prostaglandins and neutrophil chemotaxis. Several eugenol derivatives have active compounds that can be developed into new medicinal compounds as an anti-inflammatory [9,10,11]. Eugenol derivative compounds that have the potential to be developed as anti-inflammatory compounds are acetyl eugenol (4-allyl-2-methoxyphenyl acetate). Adding an acetyl group to eugenol will make the structure of the eugenol compound larger which can increase the selectivity and inhibition of the COX-2 enzyme[12,13].

Eugenol has been shown to have associated anti-inflammatory activity with the ability to inhibit the formation of prostaglandins in the pathway cyclooxygenase 2 (COX-2) [14]. Nonetheless, eugenol has a structure of relatively small molecules and less bulk, so it can also inhibit the cyclooxygenase-1 (COX-1) enzyme. Therefore, it is necessary to modify the eugenol structure by adding an acetyl group to produce acetyl eugenol (4-allyl-2-methoxyphenyl acetate). The addition of an acetyl group via esterification reaction will make the structure of the eugenol compound bigger to increase the selectivity of formation inhibition prostaglandins on the COX-2 pathway and can increase the activity of its anti-inflammatory [15].

This research is based on esterification, a reaction between an alcohol and a carboxylic acid derivative using a NaOH catalyst assisted by ultrasonic waves. The use of ultrasonic has been proven to reduce reaction time and increase yield, and it is easy to react with the green chemistry method [16]. This research's novelty is using ultrasonic waves (sonochemical) based on green chemistry in synthesizing the target compound and using a DCC coupling reagent (Decyclocarbodiimide) which is expected to support the reaction support. The synthesized compounds obtained were structurally elucidation using FTIR, ATR, and GC-MS. The anti-inflammatory activity of acetyl eugenol compounds in inhibiting protein denaturation was tested using Bovine Serum Albumin (BSA) in vitro. The percentage inhibition of protein denaturation was measured using the % inhibition formula.

METHODS

Tools

The equipment and instruments used in this study were glassware, analytical balance, plastic basin, sonicator, FT-IR spectrometer (Agilent Technologies), GC-MS, melting point apparatus, sets of distillation apparatus, Buchner funnel, and silica TLC plates.

Materials

The materials used in this study were eugenol for synthesis (pa), acetic acid anhydride (pa), sodium hydroxide 10%, DCC, Bovine serum albumin, diclofenac sodium, THMA, ethanol, etc.

Synthesis of Acetyl Eugenol

The acetyl eugenol synthesis used the procedure based on slight modification [17], 0.0323 mol of eugenol was added to Erlenmeyer, and 0.25 mol of 10% sodium hydroxide was added. The mixture was put in a sonicator for 15 minutes and heated at 60^0C. Next, 0.0974 mol acetic anhydride was reacted with DCC, added to the mix, and sonicated with time variations (60, 80, and 100 minutes). The compound formed was extracted with chloroform twice, each 20 mL of chloroform, and then the chloroform phase was taken. Store in the refrigerator until the temperature is less than 10°C.

The synthesized compound was purified by extraction using cold 5% sodium hydroxide in a separatory funnel twice with 10 mL each to form two phases, the chloroform phase and the oil phase. Then, the chloroform phase was taken. Next, a water bath heated the chloroform phase at 120°C for 45 minutes. Finally, the chloroform is allowed to evaporate, the synthesized compound is obtained as solids, and the yield is calculated.

Anti-Inflammatory Activity Test In Vitro

In vitro anti-inflammatory test was carried out by testing the activity of the synthesis results against denaturing Bovine Serum Albumin (BSA). A total of 5 ml 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 [18].

Each solution was vortexed and then incubated for 30 minutes at room temperature (27ºC). After that, it was heated for 5 minutes at 72ºC and then left at room temperature (27ºC) for 25 minutes. The turbidity was measured with a UV-Vis spectrophotometer at 660 nm. The following formula can calculate the percentage inhibition of BSA denaturation:

% inhibition =

$$\frac{Abs\ kontrol\ negatif - Abs\ sampel}{Abs\ kontrol\ negatif} \text{ x } 100\% \qquad (1)$$

RESULTS AND DISCUSSION

Acetyl Eugenol was synthesized in this study through esterification between an alcohol and a carboxylic acid derivative. The structure of eugenol contains an -OH group attached to a benzene ring [15] . Based on Figure 1 and Figure 2, Acetyl eugenol was synthesized by reacting eugenol with an acetic acid anhydride. The esterification reaction process requires a long time without a catalyst; a catalyst in acetylation aims to speed up the reaction. The catalyst used is NaOH because of its alkaline nature. It can be used in acyl nucleophilic substitution that

converts weak into stronger nucleophiles so that the reaction runs fast and the yield is also significant. The ultrasonic waves assist this reaction.

Sonochemical methods have become an alternative to green chemistry in this century because this method is straightforward, efficient, have high yields, short time, and is environmentally friendly [16,19,20]. It also can prevent volatile and toxic solvents by using harmless chemicals [21]. Moreover, this method is essential in synthesizing organic compounds and the pharmaceutical industry [19].

Using a sodium hydroxide catalyst will cause the formation of a nucleophile, the eugenol ion, so the reaction can run faster and more effectively. In addition, it increases nucleophilicity resulting in the attack of the C carbonyl atom on acetic acid anhydride can take place more easily; It is hoped that the resulting yield will be higher.

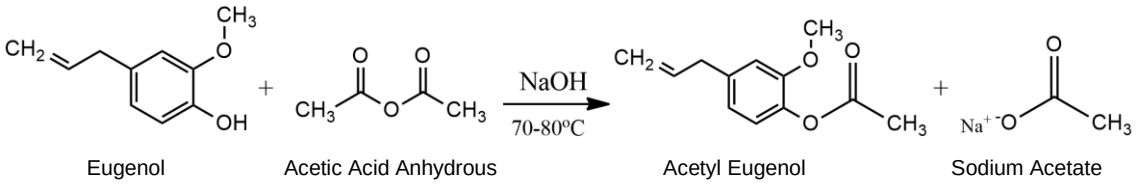

Figure 1. The reaction of Eugenol and Acetic Acid Anhydride

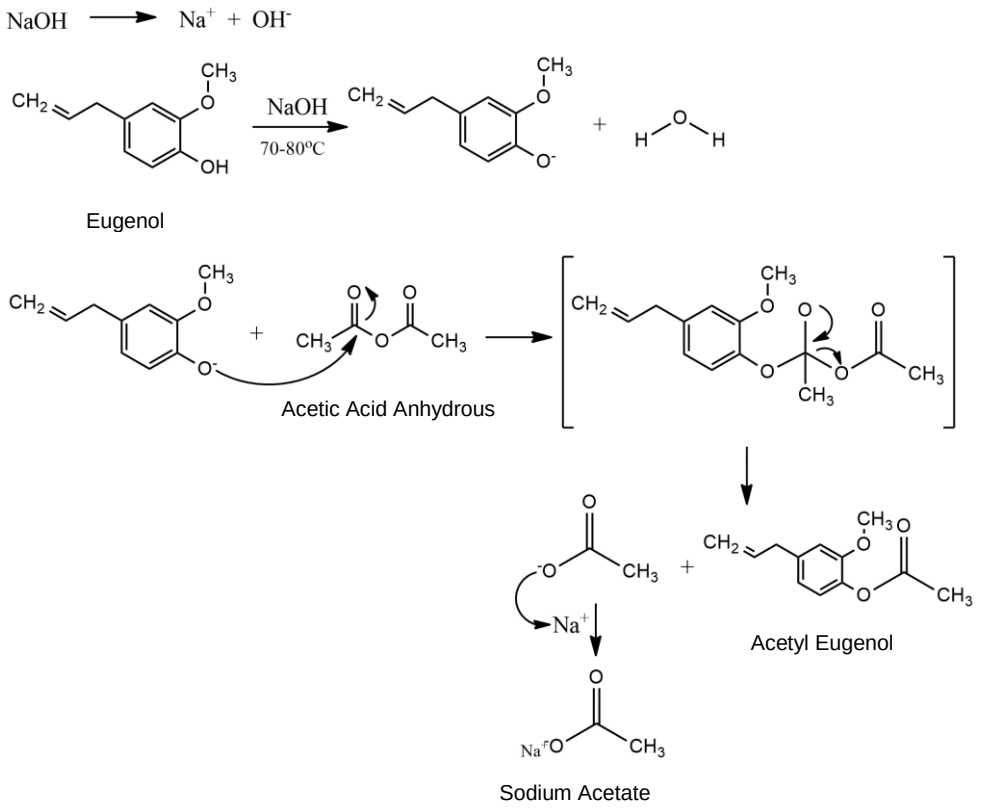

Figure 2. Reaction Mechanism of Acetyl Eugenol

The results of the structural elucidation of the synthesized compound using infrared spectroscopy in Figure 3 showed an ester functional group in the synthesized compound, which was not found in eugenol. It indicates that the synthesized compound is different from the starting material. The spectra in the image also show that the functional groups present in acetyl eugenol are found in the synthesized compound. Therefore, it further strengthens the notion that the synthesized compound is acetyl eugenol, the target compound.

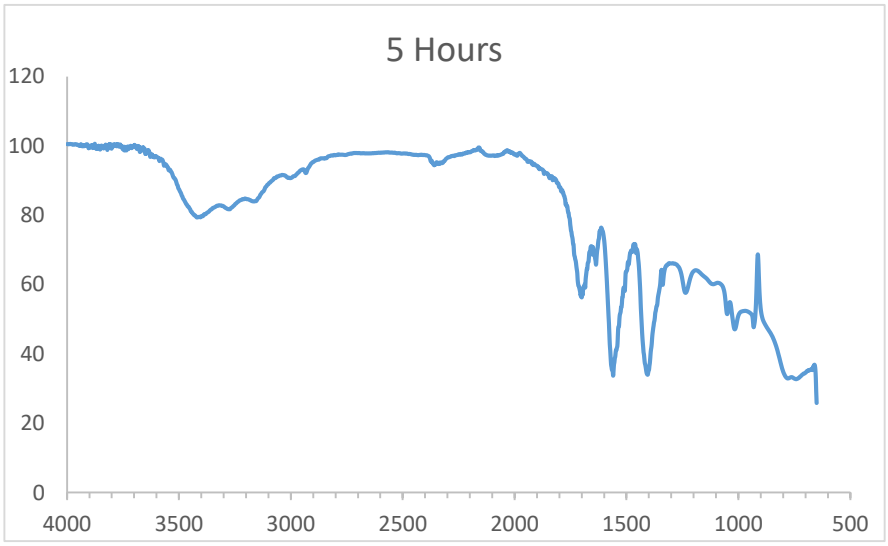

Figure 3. FTIR spectrum of synthesized compounds

Based on FTIR in Figure 4. at 3405 cm^{-1}, it indicates the presence of an O-H group (free phenol) present in eugenol. It is a functional group that distinguishes eugenol and acetyl eugenol. The presence of an alkene group (C=C) aliphatic is indicated by moderate absorption at 1405 cm^{-1}. The presence of absorption at 1560 cm^{-1} provides information that the compound being analyzed is an aromatic compound with a C=C aromatic bond. The presence of the C-O ether group is indicated by absorption at 1301 cm^{-1}. A sharp absorption at 1700 cm^{-1} indicates the presence of a C=O ester bond in the ester group [22].

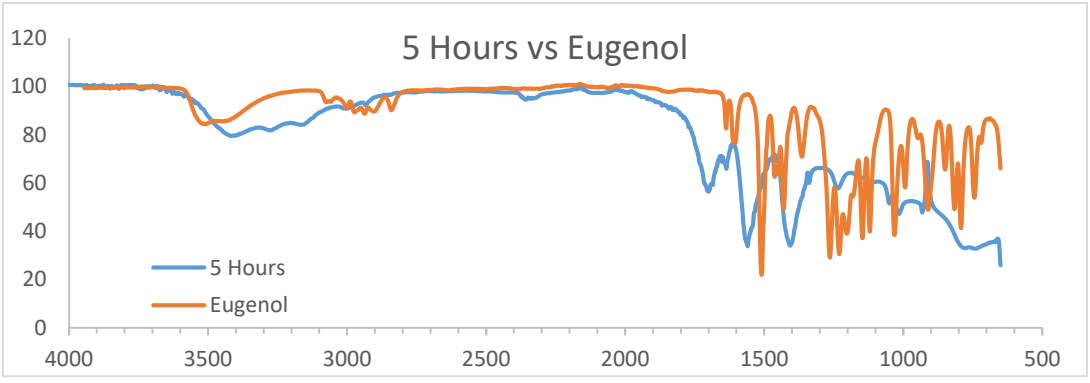

Figure 4. Overlay FTIR Spectrum Eugenol vs Acetyl Eugenol

Based on Figure 5 and Figure 6 Gas chromatography test results From the spectra and fragmentation pattern in the image. It can be concluded that the synthesized compound in the mass spectra in the picture has a molecular ion with m/z = 206 [23], according to the molecular weight of acetyl eugenol of 206 g/mol. On the other hand, the most stable ionic fragment, 37 has a molecular weight of m/z = 164, which is the breakdown of the acetyl-eugenol ester bond.

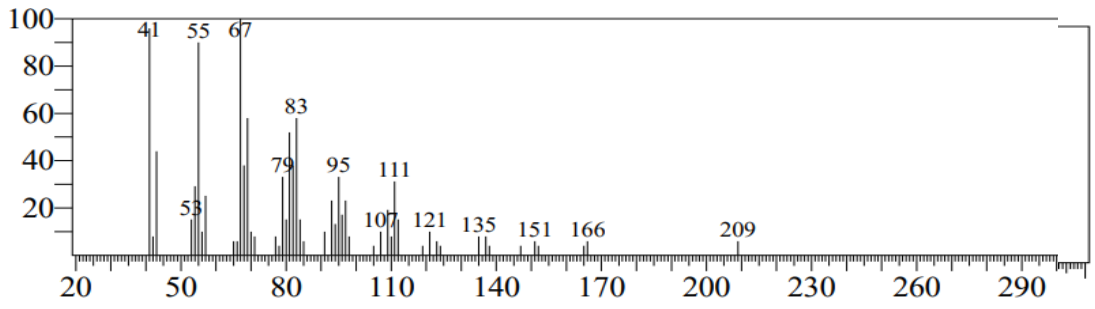

Figure 5. Mass spectra of acetyl eugenol

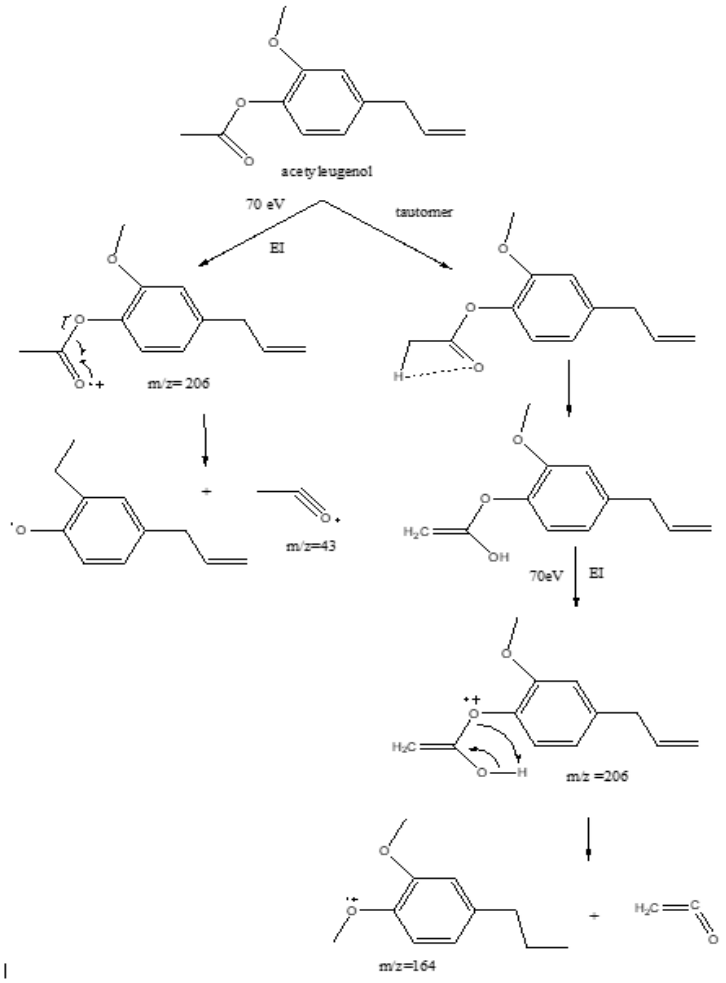

Figure 6. Fragmentation of Acetyl eugenol

Anti-Inflammatory Activity In-vitro

In this study, in vitro anti-inflammatory activity was tested on a synthesized compound using the Bovine Serum Albumin (BSA) method by observing the effect of Protein denaturation inhibition. Based on Figure 7, The measurement of BSA was performed to eliminate live specimens in the drug development process. As a positive control, diclofenac sodium has a denaturation protein inhibitory activity of 92.00% at 400 ppm. While the synthesized compound, acetyl eugenol at 400 ppm, got 32.20% inhibitory activity. The results showed that protein denaturation's inhibitory effect increased with the concentration of acetyl eugenol[24].

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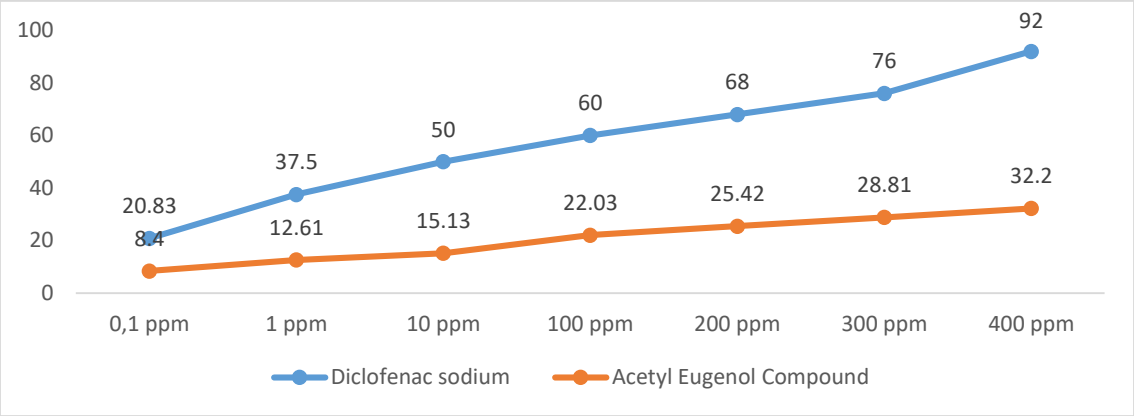

Figure 7. Concentration of Acetyl eugenol

CONCLUSION

This research synthesised acetyl eugenol through a phenol esterification reaction using sonochemical methods. This compound has the potential of anti-inflammatory in vitro with the % inhibition 32.20% at 400 ppm. The results have shown that the synthesized compound can be used as an anti-inflammatory. The FT-IR ATR and GC-MS also confirmed that the synthesized compound was acetyl eugenol with a producing a yield of 32.75%.

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