Optimization of mole ratio for synthesis reaction of Ketoprofen Eugenol Ester

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Abstract. Ketoprofen is a NSAID which often used, but has been shown to induce gastrointestinal irritation. The side-effect arises because of the presence of carboxylic acid groups. The carboxylic acid groups can be changed by esterification reaction between ketoprofen and eugenol using an acid catalyst. This study was purposed to find out the effect of mole ratio of ketoprofen : eugenol on the synthesis Ketoprofen Eugenol Ester (KEE). The mole ratio of ketoprofen : eugenol which used in the study were 1:3 ; 1:4 ; 1:5 ; 1:6 ; 1:7 ; 1:8. The synthesis was done by heating up ketoprofen, eugenol, and HCl as the catalyst. The results of synthesis were characterized using TLC, FTIR, UV-Vis Spectrophotometer, and HPLC. Then, the data was analyzed statistically using One Way ANOVA (SPSS). The result of sample analysis using TLC showed the Rf value ±0.07. The FTIR analysis indicated that there were peak C=O ester and C-O ester. The highest yield value, absorbance of UV-Vis spectrophotometer and peak area of HPLC were found in the mole ratio 1:6. The One-Way ANOVA statistical analysis indicated that there was a significant difference between the variations of mole ratio which were used.

1. Introduction
Ketoprofen (aryl propionic acid derivative) is a Nonsteroidal Anti-Inflammatory Drug (NSAID) that has been widely used to treat analgesic, antipyretic and anti-inflammatory [1]. Ketoprofen has side effects such as gastrointestinal irritation. Gastrointestinal irritation arises because of the presence of carboxylic acid group in the structure that irritant on the gastrointestinal and also ketoprofen inhibit the cytoprotective COX-1[2]. One way that can be used to reduce gastrointestinal irritation is to change the carboxylic acid group with an esterification reaction [3].

Eugenol is a volatile compound extracted from Eugenia caryophyllata Thunb., which has analgesic, antipyretic, anti-inflammatory, anti-ulcer, anti-bacteria and anti-oxidation effect [4]. Eugenol has a hydroxyl group that can be conjugate with ketoprofen. Therefore, based on prodrug principal, ketoprofen and eugenol can be combined into ketoprofen eugenol ester or KEE [2]. Eugenol is selected because it has a synergic effects with ketoprofen and reduced gastrointestinal irritation [5].

KEE can reduce side effect gastrointestinal irritation and increased therapeutic effect of ketoprofen [2]. KEE can be obtain with esterification reaction of ketoprofen and eugenol with acid catalyst (HCl). Esterification reaction ketoprofen and eugenol is a reversible reaction, where reaction can be to the right or towards the products and to the left or towards the reactants [6]. Maximum yield of KEE can
be obtained by changing the reaction condition must be to the right. To change reaction to the right, the number of reactant can be increased [7], so the appropriate mole ratio of ketoprofen and eugenol is needed. This study was aimed to optimize the mole ratio of ketoprofen and eugenol on the synthesis of ketoprofen eugenol ester to achieve maximum yield (%) of KEE.

2. Experimental
2.1. Materials
Eugenol (PT. Indesso), Ketoprofen (PT. Kimia Farma), analytical grade solvents like hydrochloric acid, methanol, n-hexane, ethyl acetate, chloroform, petroleum ether, TLC silica gel 60 F$_{254}$ (Merck), sodium bicarbonate, aquadest, analytical balance (Mettler Toledo), micropipette (Thermo), UV-Vis spectrophotometer (Genesys™), FTIR Spectrometer (Shimadzu FTIR-8201 PC), HPLC (Waters) with a UV-Vis detector and a C-18 column.

2.2. Methods
2.2.1. Synthesis of the KEE
According to Rifqi [8] a mixture of ketoprofen 2.55 g, eugenol 6.3 mL and hydrochloric acid 1 mL was refluxed for 8 hours at a temperature of 50 ± 5 ºC. The mixture was added water and decanted to separate the organic layer. The organic layer was washed with NaHCO$_3$ solution until the pH becomes neutral, followed by water 15 mL and the mixture separated with liquid-liquid extraction (LLE). The crude oil distilled out under reduced pressure for purification. The oil was then dissolved in petroleum ether and dried in an oven overnight. Synthesis was carried out on the mole ratio of ketoprofen: eugenol at 1:3 ; 1:4 ; 1:5 ; 1:6 ; 1:7 ; 1:8.

2.2.2. Characterization of KEE
2.2.2.1. Thin Layer Chromatography (TLC)
TLC plates were prepared by silica gel 60 GF$_{254}$ as stationary phase. Synthesis sample 0.1 g was dissolved up to 10 mL with methanol. Using micropipette, about 2 µL sample and standard were loaded into plates. Ketoprofen standard, eugenol standard and sample analysis were developed in TLC plate with mobile phase n-hexane: ethyl acetate: chloroform (2:5:1 v/v) [2]. The chromatograms were observed under UV lamp at 254 nm and photographed. The Rf value was obtained by using the following formula:

$$R_f = \frac{\text{distance traveled by the center of a spot}}{\text{distance traveled by the solvent front}}$$

2.2.2.2. Fourier-Transform Infrared Spectroscopy (FTIR)
Synthesis sample dropped into KBr plates and stacked with other plates [9], then sample analyzed and FTIR spectra were obtained.

2.2.2.3. UV-Vis Spectrophotometer
A 0.1 g synthesis sample was dissolved up to 10 mL with methanol then dilution was carried out to obtain a 10 ppm concentration solution. Absorbance then was analyzed by UV-Vis spectrophotometer at 261 nm [2].

2.2.2.4. High Performance Liquid Chromatography (HPLC)
Synthesis sample 0.1 g was dissolved up to 10 mL with methanol then dilution was carried out to obtain a 100 ppm concentration. HPLC analysis was carried out with C18 as stationary phase and methanol: water (65:35 v/v) as mobile phase. The measurements used were flow rate 1 mL/minute, UV-Vis detector and sample injection volume was 20 µL.
2.3. Data Analysis
Statistical analysis was carried out for absorbance data UV-Vis spectrophotometer using analysis of variance (ANOVA) test, followed by LSD test for determining level of significance. If P-value < 0.05 were considered statistically significant.

3. Results and Discussion
3.1. Synthesis of KEE
The conjugation of ketoprofen and eugenol have many advantages were to synergistic analgesic and anti-inflammatory effects and reduced gastrointestinal irritation [2]. Ketoprofen has carboxylic acid group that can be esterification with hydroxyl group of eugenol with HCl as catalyst, the esterification reaction KEE was reversible (Figure 1). A carboxylic acid can be esterified using excess alcohol that may be reaction shifts to the right or towards the product [6].

![Figure 1. Esterification reaction ketoprofen and eugenol with HCl as catalyst.](image)

Synthesis of KEE carried out several variations of mole ratio of ketoprofen and eugenol to obtain maximum yield (%), the results of the calculation of the average yield increased from KEE 3 to KEE 6 and dropped to KEE 7 and 8 (Table 1). The maximum value of the yield at KEE 6 is 65.60% and minimum value at KEE 3 is 31.85%.

| Sample     | Average Yield (%) ± SD |
|------------|------------------------|
| KEE 3 (KTP : EUG = 1:3) | 31.85±3.58             |
| KEE 4 (KTP : EUG = 1:4) | 38.93±1.58             |
| KEE 5 (KTP : EUG = 1:5) | 40.50±8.50             |
| KEE 6 (KTP : EUG = 1:6) | 65.60±2.91             |
| KEE 7 (KTP : EUG = 1:7) | 53.67±1.92             |
| KEE 8 (KTP : EUG = 1:8) | 50.80±2.35             |

3.2. Characterization of KEE
3.2.1. Thin Layer Chromatography (TLC)
Synthesis sample were analyzed using TLC with a silica gel 60 F_{254} as stationary phase and a n-hexane: ethyl acetate : chloroform (2:5:1 v/v) as mobile phase [2]. The mobile phase use of a solvent mixture aims to obtain optimal separation [10]. Before elution process, chamber was developed with mobile phase.
Figure 2. TLC profile of synthesis sample, eugenol standard (1), ketoprofen standard (2) under UV lamp at 254 nm

Table 2. Rf value of TLC sample, standard ketoprofen and standard eugenol

| Sample            | Rf 1 | Rf 2 | Rf 3 |
|-------------------|------|------|------|
| Eugenol           | -    | -    | 0.9  |
| Ketoprofen        | 0.51 | -    | -    |
| KEE 3 (KTP : EUG = 1:3) | -    | 0.70 | 0.85 |
| KEE 4 (KTP : EUG = 1:4) | -    | 0.70 | 0.89 |
| KEE 5 (KTP : EUG = 1:5) | -    | 0.70 | 0.89 |
| KEE 6 (KTP : EUG = 1:6) | -    | 0.71 | 0.89 |
| KEE 7 (KTP : EUG = 1:7) | -    | 0.69 | 0.86 |
| KEE 8 (KTP : EUG = 1:8) | -    | 0.69 | 0.85 |

Visualized TLC spot under UV lamp at 254 nm (Figure 2) with average Rf (2) value 0.70 was identified as KEE (Table 1). According to Dhokchawle et al. [2], Rf value of KEE was 0.71 with n-hexane: ethyl acetate : chloroform (2:5:1 v/v) as mobile phase that same with this study.

3.2.2. Fourier-Transform Infrared Spectroscopy (FTIR)
The synthesized samples were analyzed using FTIR (Fourier-Transform Infrared Spectroscopy) to determine the functional groups of the compound. Different bonds (C-C, C = C, C-O, C = O, O-H) in organic compounds have different vibration frequencies and can be detected by identifying frequencies as absorption in infrared spectra [11]. The result of FTIR spectra (Figure 3, Table 2) show that there are ester groups in the synthesized samples which are characterized by peaks at wavenumber of carbonyl C=O ester (1750-1725 cm\(^{-1}\)) and C-O ester (1300-1100 cm\(^{-1}\)) [12]. The presence of an ester group indicates that the esterification reaction ketoprofen with eugenol was successful.
Table 3. Result of analysis sample with FTIR

| Sample            | KEE 3 | KEE 4 | KEE 5 | KEE 6 | KEE 7 | KEE 8 |
|-------------------|-------|-------|-------|-------|-------|-------|
| C-H aromatic      | 3065  | 3062  | 3065  | 3062  | 3073  | 3068  |
| C-H aliphatic     | 2975  | 2937  | 2937  | 2937  | 2937  | 2938  |
| C-H aliphatic     | 2841  | 2910  | 2910  | 2909  | 2909  | 2908  |
| C=O ester         | 1735  | 1729  | 1735  | 1729  | 1729  | 1730  |
| C-O ester         | 1274  | 1270  | 1273  | 1269  | 1268  | 1271  |
| C=C aromatic      | 1602  | 1600  | 1603  | 1601  | 1601  | 1604  |
| C-H aromatic      | 3065  | 3062  | 3065  | 3062  | 3073  | 3068  |

Figure 3. FTIR spectra sample

3.2.3. UV-Vis Spectrophotometer

Synthesis sample was calculated absorbance at the maximum wavelength of KEE at 261 nm [2]. Analysis of samples using UV-Vis spectrophotometer showed that absorbance at 261 nm with KEE 3 to KEE 6 would increase, while in the absorbance of KEE 7 and KEE 8 decreased (Figure 4). Absorbance are directly proportional to the quantity of solute according to Lambert-Beer equation, if the absorbance value of the same compound increases, the concentration of the compound also increases [9]. The maximum absorbance value was 0.308 at KEE 6 which also shows the greatest KEE concentration, while minimum absorbance 0.19 was KEE 8 that mean KEE 8 has minimum concentration of KEE.
Absorbance data from different sample were compared by one-way analysis of variance (ANOVA). All result showed that significant difference between sample because have p value <0.05. Statistically it can be said that if mole of reactants changed caused significantly different results of reaction product.

3.2.4. High Performance Liquid Chromatography (HPLC)
Analysis by HPLC to confirm the maximum KEE in the sample. The decrease area HPLC indicated decrease of concentration of compound [10]. According to Figure 5, the highest area at KEE 6 that mean KEE 6 has the highest concentration of KEE. These results are consistent with the results of the analysis using UV-Vis spectrophotometer.

Figure 5. Result of area sample with HPLC
The results of sample analysis at variance mole ratio of ketoprofen : eugenol from 1:3 to 1:6 increased. This is accordance with the Le Chatalier principle that the reaction rate is proportional to the concentration of the reactant, if the reactants are added, the equilibrium of the reaction will shift to the right so that the number of products increases [13]. While at the ratio of ketoprofen : eugenol at 1:7 and 1:8 the value of KEE decreased. It can be said that in the ratio of 1:6 the equilibrium of the reaction has been reached. If the reaction has reached equilibrium, the addition of reactants will not have an impact on the number of reaction results [13]. The decrease can occur due to the hydrolysis reaction of the ester to the initial product. Excessive use of eugenol can produce excess water content that can hydrolyze KEE [14].

4. Conclusion
The optimum of ratio mole of ketoprofen : eugenol in the synthesis KEE was 1:6, with 65.60% of yield, 0.71 as Rf value with n-hexane: ethyl acetate : chloroform (2:5:1 v/v) as mobile phase, peak at 1729 cm⁻¹ wavenumber of carbonyl C=O ester (1750-1725 cm⁻¹) and peak at 1268 wavenumber of C-O ester (1300-1100 cm⁻¹) of FTIR spectra, 0.308 of absorbance at 261 nm, and 321,402 of area HPLC.

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