Optimization of Pre-treatment Method in Shikimic Acid Extraction from Palm Oil Mill Effluent (POME)

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Abstract: The global pandemic spread of influenza like bird flu and swine flu are forcing a bigger production of anti-influenza drug called Oseltamivir phosphate. The drug is made by synthesizing a compound named shikimic acid. Although the demand of the drug increases, the source of shikimic acid material is limited. Meanwhile, palm oil mill effluent (POME), a waste generated from palm oil industry is provenly rich in phytonutrients. This research aims to improve the shikimic extraction process from palm oil mill effluent by pre-treating the POME. Three pre-treatments were done on the POME which are solvent-extraction method, sedimentation method, and combination of solvent-extraction and sedimentation method. Sedimentation used the centrifugal principle and solvent-extraction used n-hexane as the solvent. Extraction of shikimic acid from pre-treated POME was done using ethyl acetate as the solvent. Detection of shikimic acid was done using TLC method and FT-IR instrument, while HPLC was used to measure the amount of shikimic acid extracted. Yield of shikimic acid obtained after solvent-extraction pre-treatment (0.0795%) was better than the sedimentation pre-treatment (0.015%). Also, the solvent-extraction pre-treatment was more efficient (87.51%) than sedimentation pre-treatment (68.15%) in removing oil and grease. However, the best result was produced from the combination of both pre-treatments.

Keywords: Shikimic Acid, Palm Oil Mill Effluent, HPLC, FT-IR, TLC, Pre-treatment

1. Introduction

Influenza is the main disease commonly found in this world, and with the spreading, it can be a pandemic disease globally. For instance, avian flu and swine flu have lately become common among any other influenza type since its lethal effect on humans that was diagnosed by swine and avian flu. This will lead to the discovery of the cure or the drugs for this disease.

Oseltamivir or Tamiflu® produced by Roche Pharmaceuticals is one of the drugs produced to treat influenza (Antiviral drugs). These drugs are made by synthesizing a substance named shikimic acid chemically. Shikimic acid is the precursor compound used to produce this antiviral drug, and it is commonly taken from Chinese star anise fruit which is the main source of shikimic acid. The Chinese Star anise (*Illicium verum*) is known to contain around 2-7% of shikimic acid. However, this type of *Illicium verum* plant is not easily cultivable. This leads to the shortage of shikimic acid source while the demand of the oseltamivir is still increasing. (Ghosh, S. *et al*. 2012)

Due to the high number of deaths and fatal effects caused by avian and swine flu, the production of the antiviral drugs is expected to be vast, yet the availability of the *Illicium* plant family is limited. This will lead to another problem, which how to find another source of shikimic acid that exists naturally in plant. On the other hand, as being stated on 2011, Indonesia was the largest palm oil manufacturer in the world surpassing Malaysia. Providing half of the world palm oil supply, the waste produced is also huge. Palm Pressed Fibers, Empty Fruit Bunches, Oil Palm Fronds, Oil Palm Trunks, palm shells and Palm Oil Mill Effluent (POME) are the example of waste produced from Palm Oil Industry.

The waste is causing major environmental issues, since it contained high value of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) especially in Palm Oil Mill Effluent (POME) if untreated. Waste management principle is made to minimize and recycle the waste, recover energy, and reduce pollutant's compound so it is safe to dispose. With the alternative uses of the waste, it is unfortunate and yet prohibited if just being directly disposed. To sustain the environment is the main reason, but also economical value could be also added since it can be used for other purposes, for example Biofuel or Biodiesel can be utilized from the waste of Palm Oil. (Kanta, Plangklang, & Subsingha, 2014)

Hence, as stated by the EP 2582654 A1 Patent, Oil Palm based extract has been reported to have high content of phytonutrients and its antioxidant activity. Various biochemicals compounds, nutrients, and polyphenol are the examples of valuable content exist in the Palm Oil. Even, it is remained excess in a very small amount in the industrial waste after going through a lot of treatment. However, on industrial level, it is quite big loss if not being recovered. Following these studies on phytochemical extraction from palm oil, it gives a great prospect in phytochemicals extraction from Palm Oil waste generated from industry.

By succeeding from previous research, Utilization of Palm Oil Mill Effluent to Obtain Shikimic Acid (Fajri, 2014), it is concluded that shikimic acid can be recovered using liquid-liquid extraction method. Moreover, research titled as Improvement in the Extraction of Shikimic Acid from Palm Oil Mill Effluent (Dewi, 2017) confirmed that optimization in the shikimic acid extraction process need to be done, because the amount of impurities is still high. Proceeding these 2 studies, further study on shikimic acid extraction from POME are considered.

It is the goals of this research to find a suitable method for shikimic acid extraction from Palm Oil Mill Effluent (POME). By utilizing POME generated from palm oil industry, negative impacts to the environment could be reduced. Moreover, economical value could be added to the waste treatment. Notably, POME could be another source of shikimic acid substance from plant-based component, since the avalaibility of *Illicium verum* is limited.

2. Research Methods

2.1. Design of Experiments

All This experiment compares different pre-treatment methods before the shikimic acid extraction takes place. Variations of pre-treatment methods were done on Palm Oil Mill Effluent (POME) to analyze the effects of the pre-treatments with the amount of shikimic acid extracted. Sedimentation and solvent-extraction are the principles used. Four pre-treatments were done on the Palm Oil Mill Effluent (POME), they were direct POME without pre-treatment, solvent-extraction pre-treatment, centrifugation pre-treatment, and combination of sedimentation and solvent-extraction pre-treatment. Output of these 4 pre-treatments then undergone the Oil & Grease, TLC, FT-IR analyses, Shikimic Acid extraction, and HPLC measurement.

2.2. Oil & Grease Analysis

Oil and Grease analysis was done to measure the amount of impurities in our sample. Before the shikimic acid extraction, oil and grease analysis was done to measure the amount of oil and grease after the pre-treatment steps. Initial content of Oil and Grease in Palm Oil Mill Effluent also measured to compare the result with the after- treatment. Hexane Extractable Material (HEM) Method (EPA-821-R-10-001) with few modifications was used to measure the oil & grease content. Gravimetric calculation was used to determine the amount of Oil and Grease removed.

2.3. Thin Layer Chromatography (TLC)

TLC paper was cut into size of 4x10 centimeters. Later, starting and finish line was drawn horizontally at 1 centimeter long from top and bottom paper. Next, TLC paper was heated at temperature of 105^{o}C on the hotplate for 10 minutes before being used. At the moment, mixture of methanol and chloroform with ratio of 1:4 was prepared and poured into TLC chamber. TLC chamber was closed so the saturation of vapor eluent in chamber achieved.

In spotting, sample must be dropped slowly in the starting line, and dried right away using hairdryer on the TLC paper. After spotting was done, TLC paper was putted into the TLC chamber inclined, so the bottom paper was soaked in the eluent, but without soaking the starting line and sample spots. After the solvent reached the finished line, TLC paper was taken out and placed under UV light. Standard and sample spots were marked. Distance between starting line to the spot and distance to the finish line were marked.

2.4. Fourier-Transform Infrared (FT-IR)

FT-IR analysis was done in Department of Chemical Engineering Laboratory in Universitas Indonesia. Two milliliters of the sample were prepared in closed vial. Shikimic acid standard was prepared in ethyl acetate with concentration of 5,500 ppm. Potassium bromide (KBr) was used in FT-IR analysis as the beam spliter.

2.5. Shikimic Acid Extraction

Ethyl Acetate was used as the solvent in the shikimic acid extraction process. Sample used were raffinate after solvent-extraction treatment, supernatant after centrifugation treatment, direct POME, and raffinate after the combination treatment, centrifuge and solvent-extraction treatment. Ratio of 3:2 was used for sample to solvent mixture. Sample with volume each of 60 ml were stirred with 40 ml of ethyl acetate for 1 hour at temperature of 30°C on the hotplate, followed by manual extraction in separator funnel for 15 minutes on the fume hood. Then the mixture was left to stand for 1 hour to separate the 2 layers. After an hour, upper fraction, or the ethyl acetate fraction, or the extract fraction was taken by separating the raffinate fraction.

2.6. High Performance Liquid Chromatography (HPLC)

Shikimic acid standard was made for 10,000 ppm in distilled water. The 0.01% of sulfuric acid in aqueous solution was used as the eluent or the mobile phase in HPLC system. Flowrate of sample was set into 1.0ml/min, while the volume of injection was 10μL. Ultraviolet (UV) detection wavelength was at 213 nm for shikimic acid. Column temperature was 30°C and the running time was set into 30 minutes.

3. Results and Discussion

3.1. Thin Layer Chromatography

Retention factor (Rf) calculation was used to confirm the presence of shikimic acid in the extract. Therefore, sample after pre-treatments a, b, c, and d has been confirmed to be contain shikimic acid. Rf values of each samples were quite the same compared with the pure Shikimic Acid standard. TLC analysis was done to obtain the qualitative data about shikimic acid in the extract. In the first TLC paper, pure shikimic acid standards, pre-treatment a's, and pre-treatment b's Rf values were 0.250, 0.243, and 0.262 respectively. While in the second TLC paper with the pure shikimic acid standards, pre-treatment c's, pre-treatment d's Rf values were 0.219, 0.213, and 0.237. Concluding mathematically and visually using UV light, the spot of shikimic acid was confirmed to be presence in each sample after each pre-treatment.

3.2. Fourier Transform Infrared Technology

Another qualitative measurement was done using FT-IR instrument. Each intramolecular bond exist in shikimic acid structure will be depicted as a dip in the spectrum. Table 1. shows the summary of the FT-IR results. In this case, shikimic acid standard was also used as the comparison with the samples. However, asterix marks as shown in the table means that the spectrum of the bond was not detectable by the computer since the amount might be very low, but the dip can be seen manually in the spectra with eyes.

Table 1. FT-IR Extract Results

Bonds	Frequency(cm-1)	Shikimic Acid std	Pre-treatment A	Pre-treatment B	Pre-treatment C	Pre-treatment D
C=O	1700-1725	√	√	√	√	√
C-O	1050-1300	√	√	√	√	√
O-H	3200-3550	√	√*	√	√	√*
C-H	2850-3000	√	√	√	√	√

3.3. Yield of Shikimic Acid

The yield of shikimic acid extracted was calculated as the Table 2 depicted. Total volume of extracts was the volume retained after the shikimic acid extraction in separator funnel. Percentage of shikimic acid in extracts was obtained from the HPLC result. As the percentage of shikimic acid and the injection volume of 10μL were known, the amount of shikimic acid in volume unit was obtained. Then, the conversion to the mass unit was calculated using the density of shikimic acid, which is 1.52g/ml. So, the final mass of shikimic acid contained in the 10μL injection volume was known. By multiplying with the volume of total extracts, the mass of shikimic acid in the total extracts can be obtained as in the table 2.

Table 2. HPLC Result for Shikimic Acid Yield.

Pre-treatments	Total Volume of Extract (ml)	Shikimic acid (mg)	Yield of Shikimic Acid (%)
1	24.8	5.616	0.016
		7.122	0.020
2	27	23.1	0.066
		32.7	0.093
3	29.2	5.681	0.016
		5.015	0.014
4	32.5	3,041.0	8.70
		2,208.0	6.32

3.4. Extract Results

Summarized extracted-results have been shown by Figure 1. Each number on x axis represents the pre-treatment done on the POME. As depicted by the figure, each pre-treatment divided into 2 sections based on the color. The impurities which are oil and grease was shown as the orange part in the bar and, the more contained in the extract result, the poor will the extract be. Reversely, if the blue section dominated the bar mostly, the better the result were produced, since it depicts the shikimic acid content in the extract. So, it can be concluded that the fourth pre-treatment produced the highest extract of shikimic acid and the lowest impurities while the no pre-treatment (1st) produced inversely. The fourth pre-treatment was significantly different than the others.

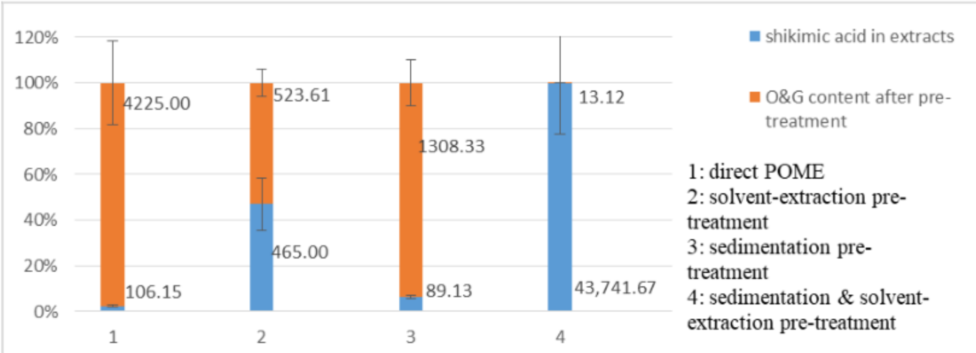

Figure 1. Extract Results from each Pre-treatments.

4. Conclusion & Recommendations

Conclusion can be made according to the data and analysis that have been collected. This chapter will discuss the conclusion of this research and the further recommendation necessary in the future.

4.1. Conlusion

- Three pre-treatments were done on the POME preceding the shikimic acid extraction, which are solvent-extraction method, sedimentation method, and combination of both methods. In conclusion, all the pre-treatments can be used to extract shikimic acid
- Solvent-extraction method is better than the sedimentation method in pre-treatment. This was implied by the efficiency in removing oil and grease in solvent-extraction with 87.51% while on sedimentation was 68.15%. Also, solvent-extraction method has better yield in extracting shikimic acid from POME (0.079%) than sedimentation method (0.015%).
- The best pre-treatment was the combination between sedimentation and solvent-extraction pre-treatment, with efficiency of oil and grease removal was 99.68% and yield of shikimic acid extracted was 7.51%.
- Ethyl Acetate can be used as the solvent to extract shikimic acid from Palm Oil Mill Effluent as showed by TLC and FT-IR result.

4.2. Recommendations

Based on the result of this research, there are still further improvements needed to achieve better result in extracting shikimic acid from Palm Oil Mill Effluent (POME). Hence, several recommendations are made for the future research and studies in this interest. These are the recommendation made according to this research:

- The recovery and the separation of the solvent from the sample need to be optimized to produce better result.
- Another method of HPLC in measuring shikimic acid extracted could be applied to produce better separation peak in HPLC.
- Further studies on POME degradation relation with the shikimic acid content needs to be done.
- Further research need to be done to achieve higher shikimic acid yield.

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