Physicochemical Properties and Antibacterial Activity of Castor Oil and Its Derivatives

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Abstract. Castor oil is vegetable oil sourced from castor seeds (Ricinus communis Linn). The main content of fatty acids in castor oil are ricinoleic acid (92%), oleic acid (3.53%), linoleic acid (2.90%), stearic acid (1.02%), and myristic acid (0.55%). Research on the antibacterial activity of castor oil and ricinoleic fatty acid has been carried out but for the K-soap and fatty acids methyl esters of castor oil have not been conducted. This research aims to produce castor oil derivatives, namely K-soap, free fatty acids (FFAs) and fatty acids methyl esters of (FAMEs) and evaluate their antibacterial activity. The results of the study include (1) K-soap (solid, white, melting point 168–175°C), (2) free fatty acids (liquid, yellow, boiling point 210°C, density 0.98 g.mL⁻¹, refractive index 1.46, viscosity 693.22 cSt, and the value of acids, saponification, and esters are 145.88, 294.52, 148.64), (3) fatty acids methyl esters (liquid, yellow, boiling point 170°C, density 0.98 g.mL⁻¹, refractive index 1.46, viscosity 27.31 cSt, and the value of acids, saponification and esters are 0.33, 392.7, 392.37). K-soap, free fatty acids, and methyl esters from castor oil have antibacterial activity against Escherichia coli and Staphylococcus aureus bacteria.

1. Introduction

According to renewal, natural resources can be classified into two, namely renewable resources and unrenewable resources. Unrenewable resources, namely oil, natural gas, and coal are very broad but still limited. The source of these chemicals will become drained with sustainable exploitation [1]. Conversely, renewable natural resources are reliable resources for fuels and chemicals in the long run [2]. This natural resource has many forms, namely carbohydrates (starch, cellulose), lignin, and triglycerides [3].

Triglycerides or oils have high potential to be developed, one of which is castor oil. Castor oil (Ricinus communis) is one of the vegetable oils with a specific fatty acid content, which contains hydroxyl groups and unsaturated chains [4]. The presence of hydroxyl groups (-OH) attached to the hydrocarbon chain in ricinoleic acid makes castor oil chemically different from other oils, especially its high viscosity and polarity. These properties make it very important for industrial production of lubrication [5], coatings, plastics, and cosmetics [6].

Castor oil has the potential as an antibacterial or antimicrobial agent [7][8]. The antibacterial activity of castor oil can be related to the content of fatty acids in it, ricinoleic fatty acid. Ricinoleic acid inhibits the growth of many viruses, bacteria, yeast, and mold, such as undecylenic acid derivatives [9][10]. The broad spectrum and strong biological activity of free fatty acids (FFAs), is its ability to kill or inhibit bacterial growth. The main target of FFAs action is cell membrane, which
interferes with the electron transport chain and oxidative phosphorylation thereby disrupting cellular energy production [11].

FFAs is a compound produced from chemical or enzymatic hydrolysis of oil or fat. The study of the antibacterial activity of a variety of pure fatty acids is carried out by taking into account the variable length of the chain and the presence or absence of a double bond C=C in the fatty acid acyl groups against Gram-positive and Gram-negative bacteria. In the context of its antibacterial activity, there are several different results between oil and free fatty acids. Tamarin oil (Tamarindus indica) is not antibacterial active [12], while free fatty acids are antibacterial against Escherichia coli and Staphylococcus aureus [13].

FFAs are already known as antibacterial and antifungal substances. In general, free fatty acids function as anionic surface agents, and the anionic surfactants are less potent at physiological logical values. The antibacterial activity of free fatty acids has a relationship with its structure. Changing the COOH group to CONMe₂ a increased the activity [14]. Besides FFA as an oil derivative, K-soap also has antibacterial activity against Staphylococcus aureus bacteria [15].

In the current research, there have been no reported of how antibacterial activity is based on polarity or ability to produce cell membrane liabilities. So, it is necessary to assess the Gram-positive and Gram-negative antibacterial activity of the oil, its free fatty acids, and its fatty acid methyl ester from vegetable oil. The broad spectrum of antibacterial activity, modes of action, and non-specific safety make it attractive as an antibacterial agent for various applications in medicine, agriculture and food preservation, especially where the use of conventional antibiotics is undesirable or prohibited [11]. This research aims to produce castor oil derivatives (K-soap, FFAs, and FAMEs), evaluate their antibacterial activity against Gram-negative bacteria (Escherichia coli) and Gram-positive (Staphylococcus aureus), and examine the relationship of polarity with their antibacterial activity.

2. Experimental Procedure

2.1. Materials, Equipments and Intrumentations

2.1.1. Material. Castor oil is obtained from an online store in Kedungkandang, Malang. The reagents and solvents used are potassium hydroxide, hydrochloric acid solution, oxalic acid solution, crystalline sodium chloride, methanol, ethanol, hexane, glycerol, acetone and chloroform, each of which has a p.a quality.

2.1.2. Equipment and Instrumentation. A set of glassware, magnetic stirrer, static, dropper pipette, stirring rod, filter paper, burette, analytical balance, thermometer, hot plate, set of melting point apparatus, autoclave, microtube, ose needle, Laminar Air Flow (LAF), bunsen, calipers, vortex, magnetic stirer, incubator, refractometer Abbe, Capillary-viscosimeter, a set of titration tools, a set of Shimadzu 8400S FT-IR spectrophotometers, and a set of GC-MS Shimadzu QP2010S.

2.2. Synthesis K-soap from Castor Oil
Castor oil (20.00 g) and 130 mL of 3 M potassium hydroxide solution were refluxed at 80 °C for 3 h and stirred with a magnetic stirrer to form two layers and there was no change anymore. This mixture is then allowed to stand and separate. Soft potassium soap is added 50 mL saturated sodium chloride solution and stirred with a magnetic stirrer until all soap is precipitated. Potassium soap deposits are washed with 20 mL distilled water and filtered with filter paper. Washed potassium soap is heated in an oven (60 °C, 2 h). Then, mashed with mortar and pastle. Potassium soap powder is characterized and identified.
2.3. Synthesis Free Fatty Acids (FFAs) from K-soap
Potassium soap (10.00 g) and 10 mL of distilled water are put into a 100 mL beaker glass then stirred with a magnetic stirrer until the K-soap is emulsified. After that, it is added dropwise 1 M hydrochloric acid solution accompanied by stirring with a magnetic stirrer until it is completely lumpy. This lump is filtered with filter paper and washed with water until the washing water is neutral. The residue obtained is left at room temperature until it melts. The liquid obtained was centrifuged at a speed of 3000 rpm for 20 minutes to separate the liquid from the remaining water and obtained residues (free fatty acids) and centrates. Free fatty acids are characterized and identified.

2.4. Synthesis Fatty Acids Methyl Esters (FAMEs) from Castor Oil
Castor oil (20.00 g), 15 mL methanol and 0.2 g of potassium hydroxide are refluxed at 60 °C for 4 h while stirring with a magnetic stirrer. The process is stopped if the mixture does not change and two layers are formed. The top layer is washed with warm water until the used washing water is neutral. The obtained mixture is then added to anhydrous magnesium sulfate to bind water. Then the mixture is filtered with filter paper to obtain water-free methyl ester filtrate. The methyl ester filtrate was characterized and identified.

2.5. Physicochemical Properties of Castor Oil and Its Derivatives
Physicochemical properties of the components are obtained by analyzing the state and color, boiling and melting points, density, refractive index, viscosity, solubility, acid value, saponification value and ester value.

2.5.1. State and Color. State and color are observed visually.

2.5.2. Boiling Point. The sample is put into a test tube that contains boiling stones and perculators. The test tube is heated to boiling and the boiling point value is constant.

2.5.3. Melting Point. The sample is placed on the glass preparation and inserted into the object into the melting point apparatus. The device is turned on and the product is observed when it begins to fuse until the product is completely fused.

2.5.4. Density. The remaining samples attached to the side of the pycnometer are cleaned. Next, the mass of the pycnometer is weighed with the sample. The mass is recorded and the density of the sample is calculated.

2.5.5. Refractive Index. Samples were dropped on the surface of the glass preparation, closed, and read the refractive index on the refractometer Abbe.

2.5.6. Viscosity. The sample is placed in the Capillary-viscosimeter and pumped with filler until it exceeds the boundary mark. The sample flow time is calculated from the upper boundary mark to the lower boundary mark that approaches the capillary tube.

2.5.7. Solubility. 3 mL of solvent is put into a test tube. The sample is added dropwise up to 0.2 mL into the test tube and shake it strongly. Observe the solubility and record the solubility of the sample in the solvent used.

2.5.8. Acid Value. 1.00 g of sample, 2.5 mL of hexane, and 4 drops of the phenolphthalein indicator were put into a 100 mL Erlenmeyer. The mixture is shaken to form a solution (homogeneous mixture). Then the mixture is titrated with 0.1 M KOH solution in water to a constant pink color solution for 20-30 seconds.
2.5.9. **Saponification Value.** 1.00 g of sample and 25 mL of 0.5 M KOH solution were put into a 250 mL three neck flask. The mixture is heated in a series of reflux apparatus at 60 °C for 1 h while stirring. Then it is cooled to room temperature and put in a 100 mL Erlenmeyer and 5 drops of phenolphthalein indicator are added. Then it is titrated with 0.5 M HCl solution until the pink turns yellow. Titration is also done on blanks.

2.6. **Identification of Castor Oil and Its Derivatives**

2.6.1. **Analysis of Infra-Red Spectrum.** Samples are mixed with KBr with a sample-KBr ratio (1: 1), pressed in a vacuum pump to form chips or pellets. Then the pellets were put into FT-IR spectrophotometry and analyzed with a frequency of 4000-400 cm⁻¹. The resulting IR spectrum is then analyzed based on a typical band at a frequency of 4000-400 cm⁻¹ which can be used to identify the functional groups contained in the sample.

2.6.2. **Analysis of Gas Chromatography-Mass Spectrometer.** The sample was injected on a GC-MS Shimadzu QP2010S device. Operational parameters are adjusted to the conditions in GC-MS. Then the chromatogram produced by the recorder and the mass spectra of each compound was observed.

2.7. **Antibacterial Activity**

2.7.1. **Media preparation.** 5 grams of instant Nutrient Agar (NA) is dissolved in 250 mL of distilled water. Then this mixture is heated while stirring until the mixture is homogeneous. 10 mL of homogeneous mixture was poured into a petri dish. Subsequently the mixture was poured into a petri dish. Subsequently the mixture was sterilized by autoclaving at 121°C for 15 minutes. Then the temperature and pressure of the autoclave are lowered, then allowed to reach ambient conditions. NA media are removed from the autoclave and left until the media solidifies.

2.7.2. **Suspension of Escherichia coli and Staphylococcus aureus Bacteria.** 10 mL Nutrient Broth (NB) was put into two test tubes. A total of 1 E. coli bacterial ose needles were inserted aseptically in the first tube and S. aureus bacteria was inserted in the second tube. Next, each mixture in the tube is rotated with vortex until a homogeneous mixture is obtained.

2.7.3. **Inhibition Test.** 6 mm diameter of the wellbore made on the media. Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacteria are inoculated into the media. Then, 20 µL of the sample is put into the wellbore. Media that have been planted with bacteria and samples incubated at 37 °C for 24 h. The diameter of the clear zone produced is measured by the calipers.

3. **Result and Discussion**

3.1. **Physicochemical Properties of Castor Oil and Its Derivatives**

Castor oil is liquid and yellow. Soap that is synthesized from oil is solid and has a white color. Changes in state during the derivatization reaction from oil (liquid, yellow) to solid and white in the saponification reaction indicate the formation of potassium soap (K-soap). Likewise, the change in state and color from the result of the acidification reaction of K-soap (solid, white) to liquid and yellow, indicates the formation of free fatty acids (FFAs). While indications of the formation of fatty acid methyl esters (FAMEs) are characterized by viscosity observed visually thinner than castor oil. The performance states and colors of castor oil and their derivatives are shown in Figure 1.

Physical properties can be influenced by intermolecular forces and molecular weights of compounds. Based on the results of physicochemical properties can be seen that castor oil has a different boiling point with fatty acids and methyl esters. Fatty acids have the highest boiling point than both because they are able to form hydrogen bonds between molecules so that the density of fatty acids is higher and requires high energy to break bonds between molecules. The difference in boiling points shows that fatty acids and methyl esters have been successfully synthesized from oil.
Figure 1. Castor oil and its derivative (a) castor oil, (b) K-soap, (c) FFAs, (d) FAMEs

Table 1. Physicochemical Properties Results of Castor Oil and Its Derivatives

| Properties           | Castor oil | K-soap | FFAs  | FAMEs  |
|----------------------|------------|--------|-------|--------|
| State                | Liquid     | Solid  | Liquid| Liquid |
| Color                | Yellowish  | White  | Yellow| Yellow |
| Boiling point (°C)   | 191        | -      | 210   | 170    |
| Melting point (°C)   | -          | 168-175| -     | -      |
| Density (g/cm³)      | 0.96       | -      | 0.98  | 0.92   |
| Refractive Index (25 °C) | 1.477  | -      | 1.462 | 1.461  |
| Viscosity (cSt)      | 520.52     | -      | 693.22| 27.31  |
| Solubility in        |            |        |       |        |
| - water              | insoluble  | emulsified | insoluble | insoluble |
| - methanol           | insoluble  | slightly | soluble | soluble |
| - ethanol            | insoluble  | slightly | soluble | soluble |
| - chloroform         | soluble    | insoluble | soluble | soluble |
| - n-hexane           | insoluble  | insoluble | insoluble | soluble |
| Acid value, AV (mg KOH/g sample) | 1.23   | -      | 145.88| 0.33   |
| Saponification value, SV (mg KOH/g sample) | 406.72 | -      | 294.52| 392.70 |
| Ester value (SV – AV) (mg KOH/g sample) | 405.49 | -      | 148.64| 392.37 |

Based on the results of physicochemical properties can be seen that castor oil has a different viscosity with fatty acids and methyl esters. Fatty acids have the highest viscosity than both because fatty acids have the highest molecular weight than oils and methyl esters so that the viscosity is greater. The difference in viscosity shows that fatty acids and methyl esters have been successfully synthesized from oil.

Based on the results of physicochemical properties can be seen that castor oil has a different density with fatty acids and methyl esters. Fatty acids have the highest density than both because they are able to form hydrogen bonds between molecules so that the density of fatty acids is higher. The difference in density shows that fatty acids and methyl esters have been successfully synthesized from oil.
The solubility of a compound is influenced by the rules of like dissolves like [16]. Based on the results of the solubility test, showed that the sequence of the nature of polarity from low to high, namely castor oil, methyl esters, fatty acids and potassium soap. The difference in solubility shows that potassium soap, fatty acids, and methyl esters have been successfully synthesized from oil. Based on the definition of acid numbers, the higher the acid numbers, the higher the amount of free fatty acids in oil or fat. Based on the results of the characterization, the fatty acids synthesized had higher acidity than oil and methyl esters. This shows that fatty acids were successfully synthesized from oil. Based on the results of synthesis, it can be seen that castor oil has the highest saponification rate. This shows that the triglyceride content in castor oil is the highest compared to fatty acids and their methyl esters because in the oil structure they have a structure with moles greater than the fatty acids and methyl esters.

Esters also show the amount of esters in oil or fat. Based on the results of physicochemical properties, it can be seen that castor oil has the highest ester number. This shows that castor oil contains quite high esters. Differences in acid numbers, saponification rates and esters indicate that fatty acids and methyl esters have been successfully synthesized from oil.

3.2. Identification of Castor Oil and Its Derivatives

3.2.1 Infra Red Spectrophotometer Analysis

Other supporting data on the success of synthesis of castor oil derivatives is the result of IR spectrophotometer identification, with IR spectra as shown in Figure 2- Figure 5.

![Figure 2. IR spectrum of castor oil](image-url)
**Figure 3.** IR spectrum of K-soap from castor oil

**Figure 4.** IR spectrum of FFAs from castor oil
Based on the interpretation of IR spectra in Figure 2–Figure 5, typical bands that distinguish the four substances summarized in Table 2.

Table 2. IR Spectrum Interpretation of Castor Oil and Its Derivatives.

| Vibration          | Castor oil | K-soap | Fatty acids | Methyl esters |
|--------------------|------------|--------|-------------|---------------|
| O-H stretching     | 3360.00    | -      | 3408.22     | 3419.79       |
| C=O stretching     | 1745.58    | 1417.68| -           | 1741.72       |
| Stretch C-H alkene | 3007.02    | 3008.95| 3010.88     | 3007.02       |

IR analysis was performed to determine the functional groups contained in castor oil and its derivatives. Based on the results of the interpretation of the IR spectra (Table 2), there are several typical absorption bands. Castor oil has a wave number 3360.00 cm\(^{-1}\) with a weak intensity indicating the existence of O-H bond stretch vibrations and wave number 1745.58 cm\(^{-1}\) with strong and sharp intensities indicating the existence of stretching vibrations of the C=O bond as esters.

The synthesized potassium soap has a wave number of 1417.68 cm\(^{-1}\) with a strong and sharp intensity indicating the stretching vibration of the C=O bond as a carboxylic salt [17]. The existence of stretching vibration C=O bonds as carboxylic salts indicates potassium soap successfully synthesized from castor oil.

Synthesized fatty acids have wave numbers 3408.22 cm\(^{-1}\) and 3388.93 cm\(^{-1}\) appear with weak intensity and width indicate the existence of O-H bonding stretch vibrations. The synthesized methyl ester has a wave number of 1741.72 cm\(^{-1}\) with a strong and sharp intensity indicating the existence of a stretching vibration C=O as an ester and a wave number 3419.79 cm\(^{-1}\) with a weak intensity indicating the existence of a stretching vibration O-H bond as an acyl group. Based on the results of the interpretation of the IR spectrum, the difference in the intensity of the O-H bond stretching vibrations
between oil, fatty acids and methyl esters shows that fatty acids and methyl esters have been successfully synthesized from castor oil.

### 3.2.2 Gas Chromatography-Mass Spectrometer Analysis

GC-MS analysis was performed to determine the content of fatty acid methyl esters synthesized. Based on GC-MS results (Table 3), the synthesized methyl esters are composed of 5 fatty acids which are indicated by the appearance of 5 peaks on the chromatogram. The abundance percentage of fatty acid content based on its fatty acid methyl ester was obtained from % Area chromatogram data. The chromatogram of FAMEs are shown in Figure 6.

![Chromatogram of FAMEs from castor oil](image)

**Figure 6.** Chromatogram of FAMEs from castor oil

Type of fatty acids based on its fatty acid methyl ester was obtained from mass-spectrometry data for each peak on a chromatogram. Analysis of mass spectrometry by EI-MS (electron impact mass spectrometry) for each peak of the five peaks of GC results obtained by mass spectra as shown in Figure 7 to Figure 11. The appropriate content of fatty acids from their fatty acid methyl esters as listed in Table 3, obtained from interpretation of each mass spectrum from nine peaks, and subsequently referred to WILEY229.LIB.

| Peak | Retention time | Relative percentage(%Area) | Fatty acid as its FAMEs | Fatty acid symbol |
|------|----------------|-----------------------------|------------------------|------------------|
| 1    | 34.997         | 0.55                        | Myristic acid          | C 14:0           |
| 2    | 38.516         | 2.90                        | Linoleic acid          | C 18:2 9c12c     |
| 3    | 38.622         | 3.53                        | Oleic acid             | C 18:1 9c        |
| 4    | 39.006         | 1.02                        | Stearic acid           | C 18:0           |
| 5    | 42.521         | 92.00                       | Ricinoleic acid        | C 18:1 9c 12-OH  |
Figure 7 Mass spectra (EI-MS) of peak 1 (methyl myristate)

Figure 8 Mass spectra (EI-MS) of peak 2 (methyl linoleate)
Figure 9 Mass spectra (EI-MS) of peak 2 (methyl oleate)

Figure 10 Mass spectra (EI-MS) of peak 2 (methyl stearate)
Based on the results of EI-MS, it can be seen that the synthesized fatty acids methyl esters are composed of 0.55% myristic acid, 2.90% linoleic acid, 3.53% oleic acid, 1.02% stearic acid, and 92.00% ricinoleic acid. The retention time, type of fatty acids, % area and fatty acid symbol summarized in Table 3.

3.3. Antibacterial Activity of Castor Oil Derivatives

Potassium soap, free fatty acids, and methyl esters which were synthesized were tested for their antibacterial activity against *Eschericia coli* as gram negative bacteria and *Staphylococcus aureus* as gram positive bacteria. The sample was dissolved in ethanol 60% and this ethanol solvent was used as a negative control. Stunted bacterial development is indicated by the formation of an inhibited zone around the wellbore. If the inhibited zone formed around the wellbore increases, the antibacterial activity produced by the sample will be even greater.

**Table 4. Diameter of Inhibition Zones**

| Type of Bacteria     | Inhibition Zone Diameter (mm) | Negative Control | K-soap | Fatty Acids | Methyl Esters |
|----------------------|-------------------------------|------------------|--------|-------------|---------------|
|                      |                               | 1 %   | 2 %   | 1 %   | 2 %   | 1 %   | 2 %   |
| *Escherichia coli*   | -                             | 18.80 | 18.90 | 15.75 | 17.45 | -     | 8.15  |
| *Staphylococcus aureus* | -                           | 14.65 | 16.90 | 13.25 | 16.35 | -     | 8.75  |

Based on the results of the inhibited zone diameter (Table 4), it can be seen that antibacterial activity of castor oil derivatives against *Escherichia coli* bacteria is higher than that of *Staphylococcus aureus*. In addition, it can be seen that potassium soap has the highest antibacterial activity compared to its fatty acids and methyl esters. This is due to differences in polarity of the three castor oil derivatives. The higher the polarity of a substance, the higher its antibacterial properties. Potassium
soap which has the highest polarity has the highest antibacterial activity followed by fatty acids and the weakest methyl esters.

4. Conclusion
Castor oil can be transformed to derivatives, i.e K-soap (solid, melting point 168–175°C), FFAs (liquid, boiling point 210°C), and FAMEs (liquid, boiling point 170°C). The antibacterial activity of castor oil derivatives against Escherichia coli bacteria is higher than that of Staphylococcus aureus. The antibacterial activity of K-soap has a higher activity than free fatty acids and higher free fatty acids than fatty acid methyl esters.

The result of this research implies that potassium salt from castor oil can be used as the control of wound, skin infection, throat infection in the skin caused by Staphylococcus aureus, while the fatty acids from castor oil have the potential as an antibacterial agent.

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