Study of antibacterial activity of *Tamarindus indica* Linn seed oil and its fatty acids

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Study of antibacterial activity of *Tamarindus indica* Linn seed oil and its fatty acids

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Abstract. The study of antibacterial activity of oil from *Tamarindus indica* Linn seed and fatty acids was conducted in this research. Oil of this plant seed was isolated by extraction and fractionation. Constituents of fatty acid in the oil identified as methyl ester fatty acids. The methyl ester fatty acid was carried out by trans-esterification with methanol/BF_3. The methyl ester was identified by gas chromatography and mass spectrometer (GC-MS). There are 13 fatty acids in the *Tamarindus indica* seed oil including octanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, eicosanoic, docosanoic, tetracosanoic, 11-octadecenoic, 11-eicosenoic, 9,12-octadecadienoic, and 9-octadecenoic acids. The transformation of tamarind seed oil into fatty acids was carried out through hydrolysis with potassium hydroxide solution followed by acidification with a hydrochloric acid solution. The physical and biological properties of the seed oil and fatty acids against antibacterial activity are reported in this paper.

1. Introduction

Tamarind (*Tamarindus indica* Linn, of the Fabaceae family) is one of the ancient plants that grow abundantly in Asian countries, including Indonesia. In Indonesia, this plant is known as Asam Jawa. Tamarin is a unique plant due to its benefit for the community, both as food and traditional medicine, by utilizing leaves, fruit, stems, especially twigs, and roots. The fruit components which are very rare to be used are seeds. Studies focusing on tamarind seeds have been carried out by researchers, especially from the fields of chemical studies and biological or pharmacological activities. Chemicals constituent investigation on *T indica* seed have been reported, especially the content of fatty acids oils and its properties [1–4] and polysaccharide contents [5–8]. The tamarind seed oil contains at least 32 fatty acids, namely 21 saturated fatty acids and 11 unsaturated fatty acids. The studies of biological or pharmacological activities of tamarind seed have been reported to have the antibacterial activity of ethanolic extract [9,10], anti-inflammatory and analgesic activities [11,12], and antioxidant activity [2]. Studies of biological/pharmacological activities of other parts of the plants (leaves, fruit, and stem bark) have also been reported [13–24]. Based on the characteristic of the chemicals structure of oil or fat as triglyceride, fatty acid was obtained from hydrolyzed oils. Thus, fatty acids widely occur in natural resources like oil, both edible oil or non-edible oil. Oils and fatty acids play an important role as nutrition substances and metabolites in the living organism [25]. Many fatty acids are known to have antibacterial and antifungal properties [27], however, the information regarding the antibacterial and antifungal properties of *Excocecaria agallocha* is limited. The relation between 30 straight-chain fatty acids and its derivatives with their bactericidal activities against 8 gram-negative and 12 gram-positive organisms has been investigated [27]. In the studies, chain length, unsaturation (cis-trans), and the functional group considered as the variable studies. The saturated fatty acid that has the most inhibitory against gram-

positive bacteria was lauric acid (C12:0), but saturated fatty acid has a lower activity than monoenoic acid (C18:1). The most inhibitory unsaturated fatty acid was dienoic derivatives (C18-2) and the others were less active. Alcohol and glyceryl esters have only activity against the gram-positive organism. Amine derivatives, in contrast with the results for fatty acids, esters, and amides, showed activity against both gram-positive and gram-negative organisms. To date, no researcher has reported the bacterial activity of fatty acids obtained from hydrolysis of vegetable oils, especially tamarind oil. This article reports the antibacterial activity of fatty acids resulting from hydrolysis (alkaline hydrolysis and continued by acid hydrolysis) tamarind oil.

2. Materials

Tamarindus indica Linn seed was collected freshly from Situbondo District–East Java Province–Indonesia. The materials used in this research were oil of tamarind (isolated from seed of *T. indica*), acetone, methanol, hexane, chloroform, potassium hydroxide, hydrochloric acid, potassium iodide, sodium thiosulphate, sodium sulphate anhydrous, glacial acetic acid, amylum, phenolphthalein indicator, bromine, aluminum foil, standard solution of McFarland, nutrient agar, liquid nutrient, pure culture of *Staphylococcus aureus* (as gram-positive bacteria) and *Escherichia coli* (as gram-negative bacteria).

3. Methods

3.1. Extraction and isolation

The seed of *T. indica* Linn (1500 g) was extracted by maceration with hexane (1L) for 24 hours. After 24 hours, hexane macerate was then filtered in a Buchner funnel, and hexane extract and residue were obtained. The additional residue was re-macerated with 1L of hexane for 24 hours for three times treatment. The Residue obtained from maceration was extracted by Soxhlet with hexane until all of the oil was extracted that was indicated by colorless of the extract. The hexane extract obtained from maceration and Soxhlet was combined. The solvent (i.e. hexane) was evaporated under reduced pressure by rotary vacuum evaporator to obtain *T. indica* Linn seed oil or tamarind oil of brownish yellow (102.30 g). The tamarind oil is then characterized by physical properties such as specific gravity, index refraction, and chemical properties (test of saponification, unsaturated degree, acid number, saponification number, and iodine number). The tamarind oil was identified by IR spectra.

3.2. Alkaline hydrolysis (saponification reaction) and acid hydrolysis of tamarind seed oil

Tamarind oil (20 g) and 50 mL potassium hydroxide saturated solution was refluxed for 3 hours in a water bath and stirred by magnetic stirrer at 80^{o}C. After reflux, the homogeneous mixture was obtained and then transferred to beaker glass. In the beaker glass, the solution was acidified (drop by drops and slowly) with a hydrochloric acid solution (1 mol.L^{-1}) and left out at room temperature until the fatty acid solidifies (indicated by red and blue litmus colors change). The fatty acid was filtered in a Buchner funnel and washed with water, and a soft solid state was produced. The product of alkaline and acid hydrolysis to tamarind oil (a mixture of fatty acids, soft solid state) was then characterized by physical properties such as specific gravity, index refraction, and chemical properties (test of saponification, unsaturated degree, acid number, saponification number, and iodine number). The tamarind oil was identified by IR spectra.

3.3. Antibacterial activity assay

Antimicrobial activity of *T. indica* seed oil and their fatty acid were evaluated by diffusion method. First, the culture of bacteria (*E. coli and S. aureus*) has been standardized with 0.5 Mc Farland reagents then inoculated onto nutrient agar plates. Second, discs of paper have been coated with standard (ampicillin and n-hexane) and a solution test (5 mg/mL). The coated discs of paper were put on bacterial cultures in the nutrient agar plates. Determination of antibacterial activity was done with measuring

inhibition zone around each disc of paper. The sample was concluded to have activity when their inhibition zone was more than 6 mm.

4. Result and Discussion

The result of physicochemical (physical and chemical) analysis of *T. indica* seed oil and its fatty acids is presented in **Table 1.** Differences on all properties were analyzed of oil and fatty acids, it showed the successful transformation of tamarind oil to fatty acids by alkaline hydrolysis and continued acidified. The properties differences are described below: that tamarind seed oil is viscous liquid while fatty acids are solid (soft solid) at room temperature and pressure indicated this transformation. Because the tamarind oil was contained saturated fatty acids more than unsaturated fatty acids [1], its fatty acids were solid (m.p. 50–55ºC). In terms of the AN (acid number) properties, AN for fatty acids (115.36 mg KOH/g) is higher than that of for tamarind seed oil (3.36 mg KOH/g, or 0.56 mg KOH/g [3]). In the cases, the tamarind oil only contained free fatty acids, while its fatty acids contained free fatty acids and fatty acids from hydrolysis result (total fatty acids**)**. Based on the differences in physical and chemical properties between tamarind seed oil and the hydrolyzed fatty acids (see **Table 1**), we strongly believe that there is a complete transformation from tamarind seed oil to fatty acids.

Table 1. Physicochemical properties of *T. indica* seed oil and its fatty acids.

Properties	Tamarind oil	Fatty acids of tamarind oil
Phase (the state at room T and P)	Viscous liquid	Soft solid
Colour	Brownish yellow	Yellowish white
Specify gravity (g/cm^3)	0.849	*NA*
Refraction index (25 ºC)	1.464	*NA*
Viscosity (cSt)	11.159	*NA*
Melting point (ºC)	*NA*	50-55
Unsaturated testing	++	++
Saponification testing	+	*NA*
Acid number, AN (mg KOH/g)	3.36	115.36
Saponification number (mg KOH/g)	280.53	114.80
Iodine number (g I_2/100 g)	111.76	53.34
Functional groups were identified on IR Spectra (vibration frequency, cm^{-1})	Showed that there of stretching vibration O–H (but very weak) 3474, C=O ester (prominent), C–O ester, and C=O carboxylic acid.	Showed that there of stretching vibration O-H (prominent, strong, and broad), C=O carboxylic acid (prominent), and C–O acid.

Note: *NA is not available. All the experimental was carried out by Duplo technique.*

Spectra produced by IR spectrophotometry analysis on tamarind seed oil and fatty acids as a result of hydrolysis are shown in Figure 1 (Figure 1a for tamarind seed oil, and Figure 1b for fatty acids), it shows a very prominent difference between the two spectra. There are bands on the wave number 3474.32 cm^{-1} (very weak) and 3422.25 cm^{-1} (prominent, strong, and broad) which is a stretching vibration frequency of O–H. In oil, this vibration appears as a result of free fatty acids content in tamarind seed oil. This strong, prominent, and broad bands of fatty acids spectrum indicate the dominant stretching vibration of hydrogen-bonded O–H, and a total transformation of tamarind seed oil triglycerides into fatty acids. Other bands frequency that supports this transformation is typical stretching vibrations of C=O and C–O at frequency 1746.22 cm^{-1} (strong, sharp) and 1713.43 cm^{-1}

(shoulder) for a carboxylic ester (tamarind oil) and the shifts at frequencies 1711.50 cm^{-1} (strong, sharp) and 1655.57 cm^{-1} (shoulder) for fatty acids. In the spectra figure, the difference in frequency bands is indicated by a dash- and solid circles.

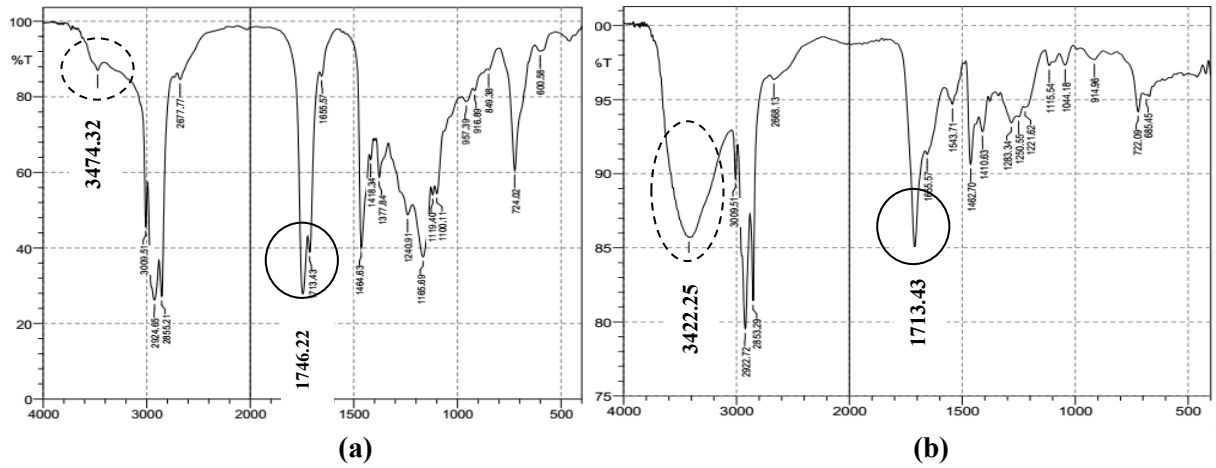

Figure 1. Infra-Red Spectra of tamarind seed oil (a) and fatty acids (b).

Based on the results of fatty acid identification analyzed as fatty acid methyl ester (FAME) with GC-MS, tamarind seed oil contains 21 saturated fatty acids and 11 unsaturated fatty acids [1]. However, the results of previous studies with the same method tamarind oil fatty acid contained 13 fatty acids, namely octanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, eicosanoic, docosanoic, tetracosanoic, 11-octadecenoic, 11-eicosenoic, 9,12-octadecadienoic, and 9-octadecenoic acids. All these fatty acids are mixed to become synergistic as tamarind seed oil fatty acids. Additionally, these fatty acids were tested for antibacterial activity and compared to the antibacterial activity of tamarind seed oil. Results of the antimicrobial activity assay of tamarind seed oil and its fatty acids against *E. coli* and *S. aureus* are shown in Table 2 and supported Figure 2.

Table 2. Results of antibacterial assay of tamarind seed oil and its fatty acids against *E. coli* and *S. aureus*

Substances	Inhibition Zone (mm)									
	E. coli (gram-negative)					*S. aureus* (gram-positive)				
	I	II	III	IV	Ã	I	II	III	IV	Ã
Tamarind oil	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Fatty acids	6.95	7.10	8.75	7.50	7.58	7.75	6.75	8.25	6.50	7.31
Ampicillin (+ control)	18.35	16.35	17.85	*NA*	17.52	17.15	17.15	17.15	*NA*	17.15
Hexane	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00

Note: Ã is average from four times treatment. Zone of inhibition 6.00 mm indicate non-active or not potential as antibacterial activity.

The result shows that fatty acids have activity against *E. coli* and *S. aureus*, but such activity is not found in tamarind oil. Antibacterial activity of fatty acids toward *E. coli* is higher than *S. aureus* but still lower than ampicillin as a positive control. The antibacterial activity against both grams positive and

gram-negative was also detected in the stem bark, fruit pulp, and leaf extracts of *T. indica* [17]. Negative activity toward *S. aureus* and *E. Coli* is detected in the tamarind because its inhibition zone diameter is 6 mm (undetected inhibition).

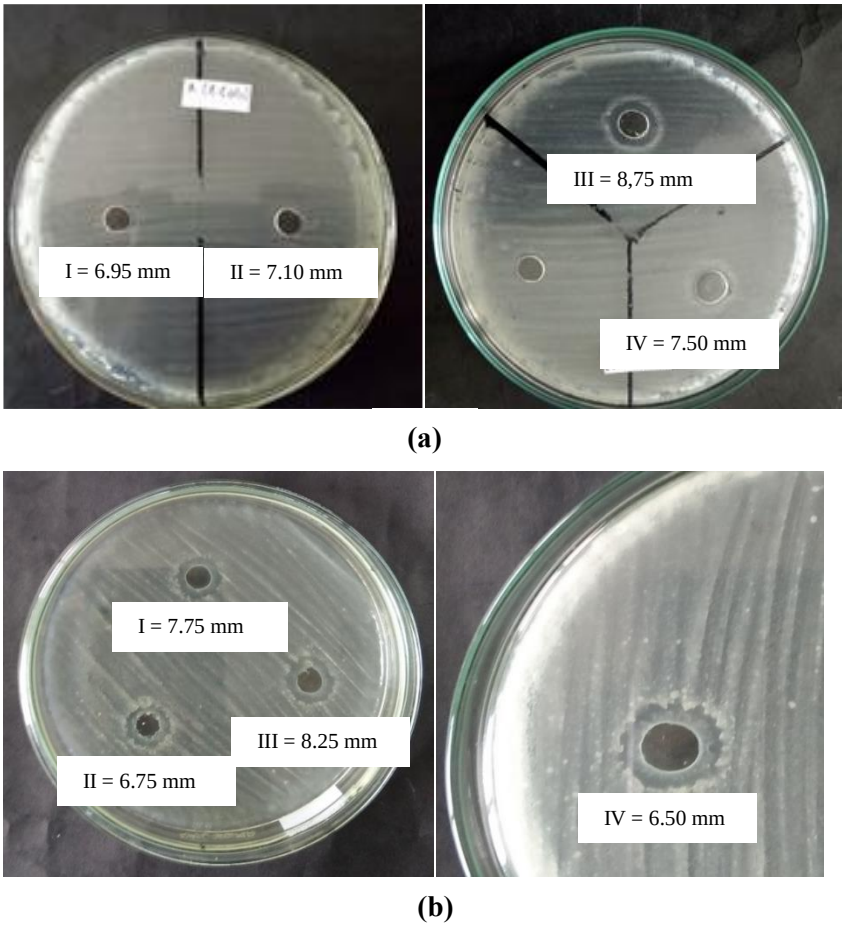

Figure 2. Antibacterial assay of fatty acids against (a) *Escherichia coli,* (b) *Staphylococcus aureus.*

Antibacterial activity of fatty acids is very diverse against gram positive and gram negative. The antibacterial activity of saturated fatty acids tends to be lower than unsaturated fatty acids. Long-chain unsaturated fatty acids, such as arachidonic acid, oleic acid, linoleic acid, linolenic acid, and palmitoleic acid show antibacterial activity. They inhibited an important component of bacterial fatty acid synthesis, which is bacterial enoyl-acyl carrier protein reductase (FabI). The most inhibited against gram-positive organisms was linoleic acid. When a straight-chain fatty acid is added with a *cis*-double bond, its activity will be increased but its *trans* isomer was not active. The addition of a second double bond further increased the toxicity of the compounds to gram-positive bacteria. A third double bond was not effective. This is in agreement with the results published by Kabara and colleagues [27].

5. Conclusion

The oil was obtained from the hexane extract of tamarind seeds. This indicates that seed is a good source of triglycerides. The main fatty acids contained by this oil are 9-octadecenoic acids, octadecanoic, dodecanoic, 9,12-octadecadienoic, octanoic, 11-octadecenoic, tetradecanoic, tetracosanoic, decanoic,

eicosanoic, hexadecenoic, docosanoic, and 11-eicosanoids. The complete transformation of oil to form its fatty acids was done by hydrolysis with potassium hydroxide solution followed by acidification with the hydrochloric acid solution. Based on physical and chemical properties, and difference of IR spectra of tamarind oil and its fatty acids, there is a total transformation of tamarind seed oil into fatty acids. The fatty acids tamarind oil is actively counter *S. aureus* and *E. coli*, but tamarind oil is inactive. However, its activity is less than ampicillin as a positive control.

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