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Profile Analysis of Fatty Acids of Tengkawang (*Shorea Sumatrana*) Oil Using GC-MS and Antibacterial Activity

Diah Gusti Riski[1], Rico Gewana Resdy Maulana[3], Edwin Permana[2], Intan Lestari[1], Indra Lasmana Tarigan[1]*

[1]Chemistry, Faculty of Science and Technology, Universitas Jambi, Jl. Jambi-Ma. Bulian KM 15 Mendalo Darat Jambi 36361, Indonesia

[2]Industrial Chemistry, Faculty of Sciences and Technology, Universitas Jambi. , Jl. Jambi-Ma. Bulian KM 15 Mendalo Darat Jambi 36361, Indonesia

[3]Department of Industrial Engineering, Faculty of Engineering, Universitas Sumatera Utara, Jl. Almamater Kampus Universitas Sumatera Utara Medan, 20155, Indonesia

Corresponding Author: indratarigan@unja.ac.id

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Abstract

The *Shorea sumatrana* (tengkawang) plant is endemic in Indonesia, especially in Kalimantan and Sumatera regions, which produces chemical diversity especially as natural drug. Specific aims to investigate both the profile analysis of fatty acid and antibacterial potential of tengkawang oil. The extract of tengkawang oil was carried out using the soxhlet extraction method. The profile analysis of fatty acid was verified by GC-MS and the antibacterial activity was evaluated using disc-diffusion method. The profile analysis of fatty acid of tengkawang oil indicated the presence of palmitic acid (17.26%), stearic acid (60.68%), oleic acid (11.98%), oleic acid chloride (1.80%), stearic acid chloride (1.86%), glycidyl stearate (1.92%), diethyl phthalate (4%), and 2-monopalmitin (0.5%). We determined the antibacterial activity by diameter of inhibition of growth zone against *Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* at a concentration of 12.5%, 25%, 50%. These were compared with standard tetracycline as positive control and DMSO was assigned negative control. It was found that the highest percentage of fatty acid in tengkawang oil is stearic acid, at 60.68%, and that tengkawang oil is an antibacterial agent with concentration optimum at 25% with more susceptibility to Gram-positive than Gram-negative bacteria.

Keywords: Tengkawang, extraction, soxhlet, fatty acids, antibacterial

INTRODUCTION

Fatty acids (FAs) are carboxylic acids functional group with long, unbranched carbon chains, with both single and double bonds. Somehow, mostly of fatty acids with double bonds carbon have cis conformation in nature, while the trans double bonds are rare (Tarigan, 2019). FAs are known as important constituent of plants and are commonly known to possess biological activity such anti-inflammatory and antimicrobial (McGaw et al, 2002). The FAs content has different chemical contents, for example, alcohols, hydrocarbons, phenols, aldehydes, esters and ketones are some of the major components of essential oil (McGaw et al., 2002; He et al., 2015). Some of those functional groups such as hydroxyl (-OH) and carbonyl (C = O) become content in plant oil which has antibacterial properties (et al., 2019). FAs are ubiquitous molecules typically found bound to other compounds such as glycerol, sugars or phosphate head groups to form lipids (Desbois and Smith, 2010). The *Shorea sumatrana* plant, from which tengkawang oil is extracted is endemic to Indonesia, especially in Kalimantan and Sumatera regions, which produces chemical diversity especially as natural drug (Hidayat et al., 2019). Tengkawang is a non-wood forest product, including in the Dipterocarpaceae family. This plant produces tengkawang oil which is high potential as a medicinal compound (Riska and Manurung, 2018). Moreover, tengkawang have been widely used by the people of Indonesia as a basic ingredient in making candles, chocolate, margarine, cosmetics, and soaps (Kusumaningtyas et al., 2012). The oil plant is a rich source of metabolites for the development of novel drugs. Stearic Acid, butyric acid and the other of short-chain fatty that mainly produced from plant that can functionally as drug compound (Keshari et al., 2019).

Tengkawang oil is produced from tengkawang fruit which is removed by the skin and then dried or lightly dried in the sun, then crushed and squeezed until the oil comes out (Kusumaningtyas et al. 2012; Hidayat et al., 2019). Singkawang oil or tengkawang oil is also known as the green butter of tengkawang seed that is ignited (Saridan, Fernandes and Noor, 2013). Traditionally, tengkawang oil is used for cooking, food seasoning, and medicinal herbs (Saridan et al., 2013; Riska and Manurung, 2018). In the field of medicine, tengkawang oil is used as an antiseptic, stimulates blood circulation, antiviral, and antitumor (Neliyanti, 2013). The Anak Suku Dalam in Province of Jambi Utilized as Dysentery Medication and Wound Medication (Kusumaningtyas et al., 2012). The specific aim this study to investigate both the profile analysis of fatty acid using Gas Chromatography-Mass Spectrophotometry and determine the antibacterial potential of tengkawang oil by the disc diffusion method.

METHODOLOGY

Materials and Instrumentals

Tengkawang seeds are taken from the Sarolangun Regency Jambi Province which is used as a sample to obtain tengkawang oil. Other chemicals were used, n-Hexane, metanol, etil asetat, butanol, n-heksana, natrium sulfat anhidrat, aquades, nutrient agar (NA), lactose broth (LB), sabouraud dextrose agar (SDA), sabouraud dextrose broth (SDB), alkohol, spirtus, suspension of bacteria *S. thypti*, *S. aeureus*, *E. coli*, *B. cereus*. There is equipment we used, such as glassware, spatulas, ovens, refrigerators, autoclaves, analytical balances, crucible pliers, Shaker Incubators, disc-diffusion plates, Gas Chromatography-Mass Spectrophotometry, and Calibration (Hidayat et al., 2019).

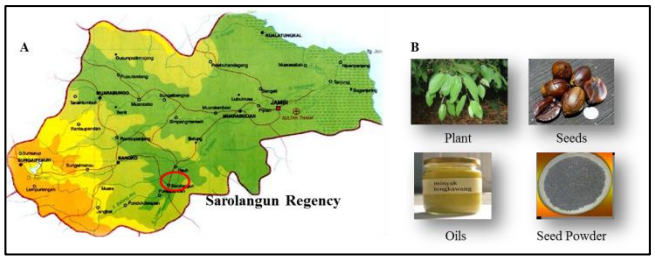

Figure 1. Sarolangun Regency, Jambi Province is one of the producers of tengkawang oil (A). Trees, Seeds and Tengkawang Oil (B).

Oil extraction and Fatty Acids Profile

Tengkawang seeds are dried and mashed in the form of fine powder. A total of 500 grams of the tengkawang seed powder were used in this study and the extract of tengkawang oil was carried out using the soxhlet extraction method using n-hexane at 67 °C for 4 hours then evavorated using rotary evavorator at 65 ^{o}C to obtained total oil extract (Rassem, Nour and R. M., 2016). GC-MS was adjusted using a Phenomenex ZB-5 column with a size of 30 mm x 0.25 mm x 0.25 mm. The condition of the injector temperature was 280 ° C and the sampling time was 1 minute.

The column temperature adjusted at 35 - 280 °C. At the initial temperature of 35 ^{o}C was held for 3 minutes, then the temperature was increased again by 10 °C/minute from 35 °C to 180 °C, then increased 30°C/minute from 180 °C to 250 °C. After reaching a temperature of 250 °C, the oven temperature maintained for 1 minute. The oven temperature was again increased by 30 °C/ minute from 250 °C to 280 °C, then at 280 °C for 7 minutes. The total program time is 29 minutes. The temperature of the detector was 280 °C, an interval temperature of 250 °C. Helium carrier gas adjusted with a constant flow rate of 2 ML per minute. Electron impact ionization is setting at 70 eV. The sample then injected 1 µL onto GC-MS instrument, dilution factor 1,000 and volume 2 mL, with an oven temperature of 650C, at an injection temperature of 2500C, and a pressure of 74.5 kPa with a total flow of 602.4 mL/min. Purge flow 3.0 mL/minute and split ratio 500.0 10 (Hartono, et al., 2012). The results of the chromatogram and component analysis from MS were then analyzed through literature studies from previous studies (Hidayat et al., 2019; Puspita et al., 2019).

Antibacterial Activity

In this study was conducted an antibacterial activity test using a modified diffusion method from previous studies (Muadifah et al., 2019), against four bacteria *S. thypti*, *S. aeureus*, *E. coli*, *and B. cereus*. The bacterial suspension is made and etched on nutrient agar medium (NA). The discs were dipped in each of three different concentrations of oil extract, 12.5%; 25%; and 50%. The DMSO as negative control while tetracycline as a positive control, then put the disk paper which has been contained sample on the agar medium. Incubation did at 37 °C with 24 h, the diameter of the clear zone appears as a clear or clean area surrounding the disc that has been dipped in oil extract. Observations were made after 24 h of the incubation period. A clear area is an indication of

the sensitivity of bacteria to antibiotics or other antibacterial agents used as test material expressed by the width of the inhibition zone diameter. The inhibition zone diameter is calculated in millimeters (mm) using calipers, then the diameter of the inhibition zone is categorized as the strength of the antibacterial power (Desbois and Smith, 2010; Kada et al., 2016).

RESULTS AND DISCUSSIONS

GC-MS analysis result

The percentage yield of tengkawang oil obtained was 40.8%. This is because the seeds used are dry seeds with very low moisture content. Tengkawang oil extraction then analysed using the GC-MS system. The results of the spectrum are analyzed shown in Figure 2. Qualitative results of oil samples indicate that tengkawang oil contains six components of fatty acids, Diethyl Phthalate, Palmitic Acid, Oleic Acid, Stearic Acid, 2-Monopalmitin, Oleic Acid Chloride, Stearic Acid Chloride, and Glycidyl Stearate. The profile analysis of fatty acid of tengkawang oil indicated the presence of palmitic acid (17.26%), stearic acid (60.68%), oleic acid (11.98%), oleic acid chloride (1.80%), stearic acid chloride (1.86%), glycidyl stearate (1.92%), diethyl phthalate (4%), and 2-monopalmitin (0.5%).

Previous research identified twenty types of sengkawang oil compounds, indicating that the bioactive compounds contained are a group of fatty acid compounds. 9-Octadecenoic acid (Z)-, methyl ester ($C_{19}H_{36}O_2$) is a fatty acid that has the highest percentage of the 19 other fatty acid compounds. Fatty acids that bind to triglycerides are carbon (C) chains with a carboxyl group (COOH) at one end that

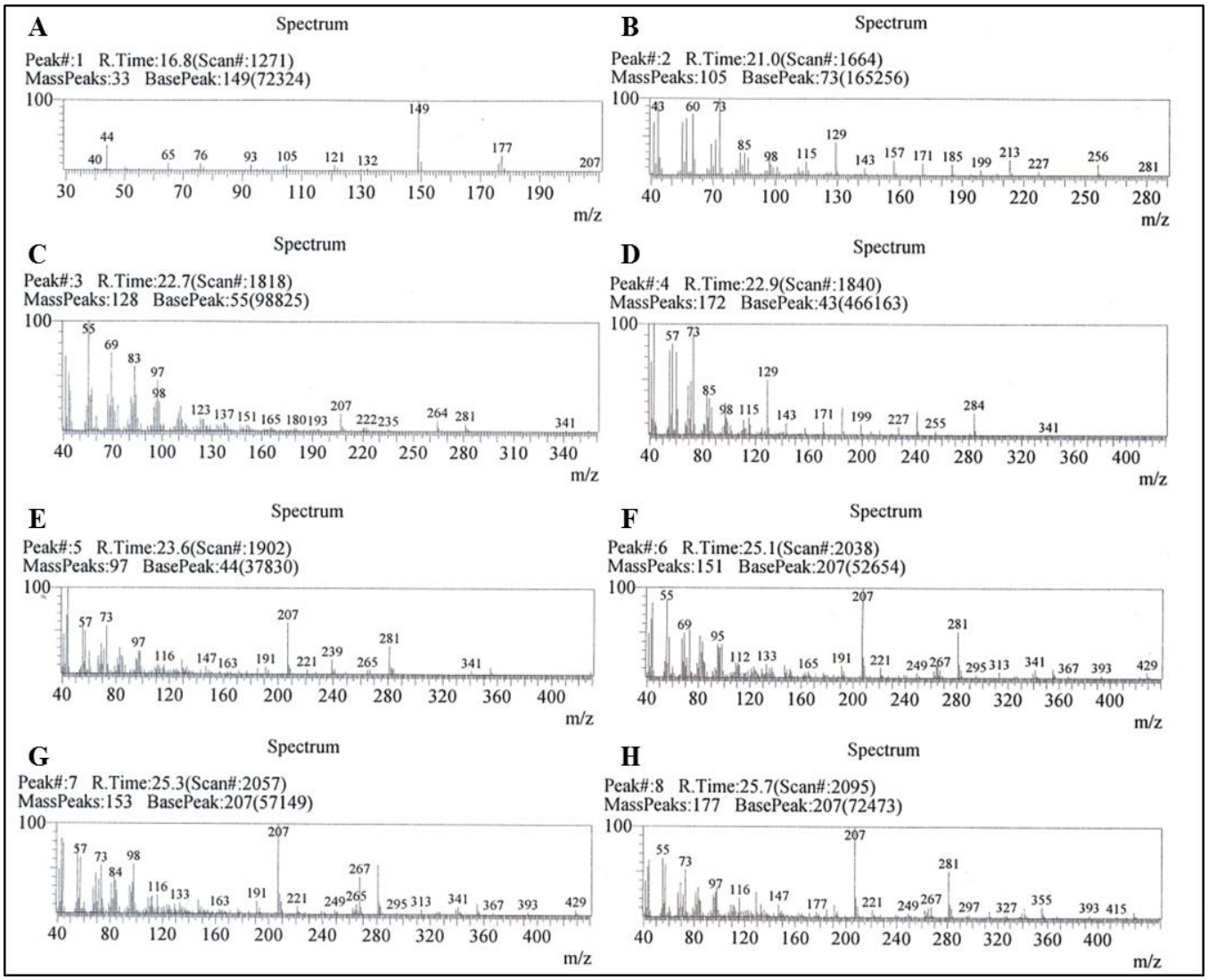

Figure 2. The mass spectrum of the tengkawang oil, (A). Diethyl Phthalate, (R.T. 16.8) (B) Palmitic Acid (R.T. 21.0), (C) Oleic Acid (R.T. 22.7), (D) Stearic Acid (R.T. 22.9), (E) 2-Monopalmitin (R.T. 23.6), (F) Oleic Acid Chloride (R.T. 25.1), (G) Stearic Acid Chloride (R.T. 25.3), (H) Glycidyl Stearate (25.7).

can react with other molecules (Hidayat et al., 2019; Puspita et al., 2019). This research was focus on fatty acid compounds and using standard compounds to identify them.

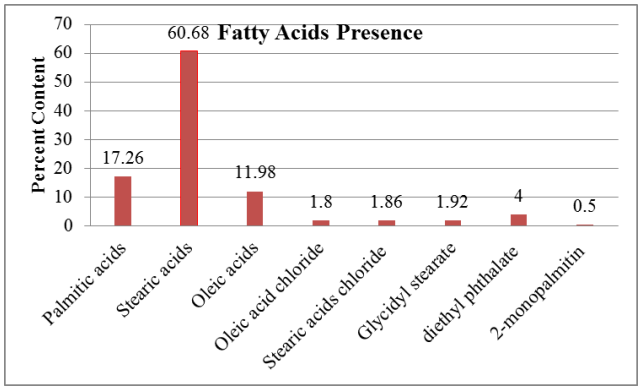

Figure 3. The profile of fatty acid tengkawang oils

Our results show that stearic acid is the major's product in tengkawang extract with 60.68% n-hexane solvent and its derivative in the form of stearic acid chloride, 1.86%, followed by palmitic acid and oleic acid with 17.26 and 11.98 respectively.

Table 1. The Profile of fatty acids of oil extracted of *Shorea sumatrana*

Peak No	Retention time (min)	Fatty Acids	Number of Carbon	Relative percentage
1	16.8	Diethyl Phthalate	$C_{12}H_{14}O_4$	4
2	21.0	Palmitic Acid	$C_{16}H_{32}O_2$	17.26
3	22.7	Oleic Acid	$C_{18}H_{34}O_2$	11.98
4	22.9	Stearic Acid	$C_{18}H_{36}O_2$	60.68
5	23.6	2-Monopalmitin	$C_{19}H_{38}O_4$	0.5
6	25.1	Oleic Acid Chloride	$C_{18}H_{33}ClO$	2.33
7	25.3	Stearic Acid Chloride	$C_{18}H_{35}ClO$	1.86
8	25.7	Glycidyl Stearate	$C_{21}H_{40}O_3$	1.92

Stearic acid and palmitic acid are saturated fatty acids, while oleic acid is an unsaturated fatty acid with one carbon atom, double bonds (Agoramoorthy et al., 2007; Tarigan, 2019). In previous studies found several fatty acid compounds in tengkawang oil such as 9-Octadecenoic acid (Z)-, methyl ester (CAS), 9-Octadecenoic acid (Z)-, methyl ester (CAS), Decanoic acid (CAS), Cyclohexaneacetic acid, butyl ester (CAS), 1,2-Benzenedicarboxylic acid, mono (2-Ethylhexyl) ester, 4,8-Decadien-3-ol, 5,9-dimethyl, Citronellyl acetate, and several other compounds (Puspita et al., 2019).

The antibacterial activity test result

Stearic acid, lauric acid, butyric acid, lactic acid, oleic acid, linoleic and so on are fatty acids methyl esters (FAME) which are known to have good antibacterial activity (Agoramoorthy et al., 2007). The results of the antibacterial activity test of tengkawang oil extract showed the optimum concentration value extracted 25% if compared to the extract concentration 12.5% and 50%. Our finding at a concentration of 25% the inhibition zone diameter of the bacteria *S. thypti* (16mm), *S. aureus* (25 mm), *E. coli* (17 mm), and *B. cereus* (22 mm), which on average are categorized as strong inhibitory antibacterial. Whereas at concentrations of 12.5% and 50% categorized as moderate inhibition, also at 50% (Table 2). The criteria of antibacterial activity refers to previous studied who classified it into: low (<5 mm), moderate (5-10 mm), strong (10-19 mm), and very strong (>20mm) (Elya et al., 2016).

Table 2. The diameter of inhibition of growth zone ethanol extract against *Salmonella thypi*

Species of Bacteria	The Diameter zone of Inhibition (mm)				
	Concentration			Control	
	12,5	25	50	Positive	Negative
Salmonella typhi	21	16	15	10	7
Staphylococcus aureus	22	27	25	17	7
Escherichia coli	8	19	17	23	9
Bacillus cereus	19	22	15	37	8

The ability of fatty acid compounds both the palmitic and stearic acid in crystallite surface displayed antibacterial activity against Gram-negative, rod-shaped *Pseudomonas aeruginosa* and Gram-positive, spherical *Staphylococcus aureus* cells (Ivanova et al., 2017). Long fatty acids can be broken down into short-chain fatty acids (SCFAs) which act as antibacterial and anti-inflammatory compounds through the mechanism of IL-6, In vivo knockdown of short-chain fatty acid receptor 2 (FFAR2) in mouse skin considerably blocked the probiotic effect of *S. epidermidis* on suppression of UVB-induced IL-6 production.

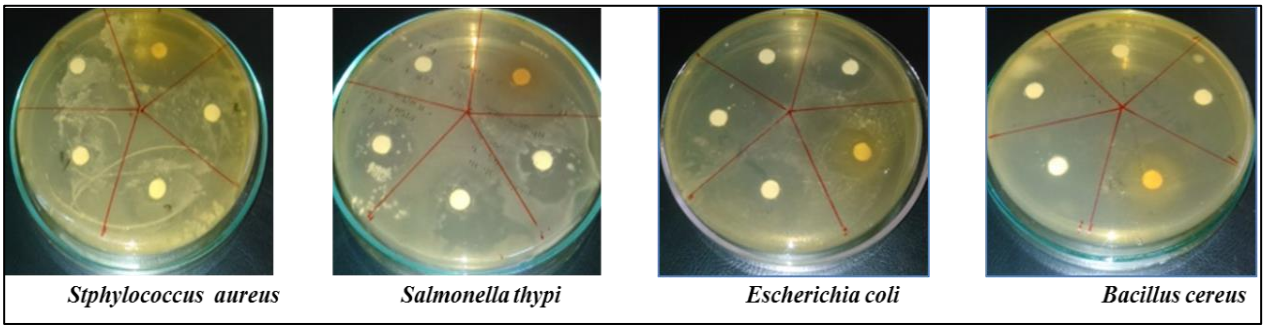

Figure 4. Disc-diffusion assay: The inhibition zone diameter oils extract against *S. thypti*, *S. aureus*, *E. coli*, and *B. cereus*

These results demonstrate that butyric acid in the metabolites of fermenting skin probiotic bacteria mediates FFAR2 to modulate the production of pro-inflammatory cytokines induced by UVB (Yang et al., 2018; Keshari et al., 2019). SCFAs act on leukocytes and endothelial cells through at least two mechanisms: activation of GPCRs (GPR41 and GPR43) and inhibition of histone de-acetylase (HDAC). SCFAs regulate several leukocyte functions including production of cytokines (TNF-α, IL-2, IL-6 and IL-10), eicosanoids and chemokine (e.g., MCP-1 and CINC-2) (Vinolo et al., 2011).

In the bacterial inhibition test, the ability of tengkawang oil extract against gram-positive bacteria (*S. aureus* and *B. cereus)* is more susceptible than gram-negative bacteria (*S. thypi* and *E. coli*). The differences in fatty acid sensitivity between Gram-positive and Gram-negative seem due to the impermeability of the outer membrane counterpart of gram-negative bacteria which is absent in Gram-positive. The outer membrane is supposed to effectively barrier against hydrophobic substances. Somehow, Gram-negative bacteria are more resistant to inactivation by medium and long-chain fatty acids than Gram-positive bacteria (Agoramoorthy et al., 2007). Our study confirm that the tengkawang oil contain higher relative percentage of Stearic acid, palmitic, and oleic acid that has potential antibacterial principle for clinical application

CONCLUSIONS

Our finding that % yield 40.8% and the highest percentage of fatty acid in tengkawang oil is stearic acid, at 60.68%. This study confirms that tengkawang oil contains a higher relative percentage of Stearic acid, palmitic, and oleic acid that has a potential antibacterial principle for clinical application. Tengkawang oil has an antibacterial agent against Salmonella thypi, Escherichia coli, Staphylococcus aureus, and Bacillus cereus at a concentration of 12.5% with the inhibition zone were 15 mm, 8 mm, 22 mm, and 15 mm respectively. These were compared with standard tetracycline as a positive control at the concentration 12.5% was 10 mm, 23 mm, 17 mm, and 37 mm.

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