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ISOLATION OF PHYTOSTEROL FROM PALM FATTY ACID DISTILLATE (PFAD) USING SOXHLET MODIFICATION

Ovalina Sylvia Br. Ginting[1]*, Effendy De Lux Putra[2], Marline Nainggolan[3] and Ahmad Gazali Sofwan Sinaga[4]

[1,2,3]Faculty of Pharmacy, University of North Sumatra.
[4]Indonesian Oil Palm Research Institute.

***Corresponding Author: Ovalina Sylvia Br. Ginting**
Faculty of Pharmacy, University of North Sumatra.

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ABSTRACT

Objective: To isolate phytosterol compounds from PFAD to obtain pure isolates. **Methods:** Saponification was used to separate the sterilized part (polar extract) containing ALB and TAG with unsabbed portions (non-polar extract) containing phytosterol. This non-polar saponification extract was isolated using modified soxhlet with silica gel binder. The identification of isolate purity was conducted using Fourier Transform Infrared (FT-IR) and Gas Chromatography Mass Spectroscopy (GC-MS). **Results:** Saponification results obtained non-polar crude extract (unsabbed portion) with a concentration of 2,683 ppm. The result identification of phytosterol isolate with FT-IR showed the similarity of functional group between phytosterol standard and isolate. However, peak appeared in the isolate at the wave number 1741 cm^{-1} showing the carbonyl group C=O. Meanwhile, identification using GC-MS showed isolate purity rate was 80% to the standard. The phytosterol content contained in the isolate was 4,035 ppm. **Conclusion:** The results of this study indicated that the obtained phytosterol isolate had a fairly good purity with a rate of 80%. The method used is also very beneficial because it can save the solvent, increase the amount of results obtained and short in time.

KEYWORDS: *Palm Fatty Acid Distillate (PFAD), phytosterol, saponification, soxhlet, FT-IR and GC-MS.*

INTRODUCTION

Palm Fatty Acid Distillate (PFAD) is produced from the deodorization process (odor) of palm oil. PFAD contains 81.7% free fatty acid (ALB), phytosterol (0.4%), squalene (0.8%), glycerol (14.4%), vitamin E (0.5%) and other components (2.2%). This component has a significant role in the health sector, including phytosterols.[1, 8, 11]

Phytosterols are also referred to as sterols which structurally similar to cholesterol, but obviously different in side chain structures. Phytosterol are powders with high melting point, stable chemicals and comparable in chemical and physical properties which is suitable to fats and oils. This compound is insoluble in water, but soluble in non-polar solvents, such as *n*-hexane, iso-octane and 2-propanol. The most common types of phytosterols are cholesterol, sitosterol and stigmasterol.[3, 7, 13]

Phytosterols are important steroids to maintain the structure of plant membranes, and form free organic compounds, phytosterols are used to maintain the balance of phospholipid membranes of plant cells, such as cholesterol in animal cell membranes. Phytosterols may inhibit the cholesterol absorption process in intestine, increase the excretion of bile salts and avoid the esterification of cholesterol in the intestinal mucosa.[7, 12-13]

Soxhlet modification using silica gel as a sample binder has been carried out by Gunawan and Hsu Ju, Y., In 2008 [6] on soybean oil and this study somehow proved that this material is more profitable due to the less solvent used, the size of the sample per batch unit and of course and shorter in time operation. Therefore, in this study, isolation of phytosterol compounds from PFAD was conducted by using soxhlet modification in order to obtain maximum results with solvent and minimalize the time use compared to other research methods conducted previously.

MATERIALS AND METHODS

Materials

Phytosterol (Sigma-Aldrich; 95%), ethanol, Ascorbic acid 1%, 50% KOH solution, *n*-hexane pa, chloroform, aquadest, PP indicator, silica gel and PFAD was derived from PT. Sintong Abadi, Kisaran, North Sumatra.

Instrument

Gas chromatography (Agilent 6890 N with autosampler) with Capillary Column: HP_5M_5 5% phenylmetylsiloxane

length 30 mx 320 µm ps, with 0.25 µm stationary phase thickness, detector: using MS, Agilent 6971 inert mass selective detector (Agilent Tech. Alto, California, USA). Injector temperature: set at 250°C. The temperature was increased 2°C / min to a temperature of 100°C and then raised to 5°C/min up to a temperature level of 290°C, kept at 290°C for 10 minutes. Carrier gas: Helium, Alphagaz. Transfer line with temperature 280°C, MS Quadrupole 150°C, MS Source 230°C. Rotary evaporator (Buchi), FT-IR (Alpha), soxhlet and analytic scale (Sartorius).

Preparation of phytosterol standard solution 400 ppm
Thoroughly weighed, the phytosterol standard of each was 0.01, dissolved with chloroform then got into a volumetric flask and accumulated up to 25 mL to obtain a standard concentration of solution of 400 ppm.[2]

Characterization of PFAD
Includes saponification number, free fatty acid and free fatty acid composition according to AOCS method [2].

Saponification
50 grams of sample were inserted into three-neck flasks of 2 L size then added 500 mL of 0.1% ascorbic acid ethanol. After that, 25 mL KOH 50% was added. all materials were refluxed for 60 minutes at temperature 60°C. The reflux results were cooled and inserted into a separating funnel, then shake strongly. 500 mL of n-hexane added, shake strongly again for 1.5 minutes, then waited until a distinct layer separateted clearly. Once the layer already formed, the top pipette was moved to other separating funnel. Those steps had to be repeated 2 times. After that, the entire solution was washed in a separating funnel (2) with 40% ethanol, shaken strongly and holded for 1 minute until two layers were formed then the bottom layer was removed. Those steps should be repeated 2 times then washed again with a warm aquadest, added 3 pounds of PP solution, shake well for 30 seconds. it was holded until 2 more layers were formed. Discard the bottom layer, then repeat the solution washing step in the separating funnel (2) until the bottom layer (aquadest) is clear. Then the pipette top layer that has been neutral was put into the evaporation flask. Furthermore, the solution was evaporated at temperature 40°C until crude non polar extract was obtained.[2]

Isolation of phytosterol using modified soxhlet
A total of 5 grams of silica gel was soaked in *n*-hexane then filtered, dried in the oven at a temperature of 100°-150°C for 1 hour. Crude extracts were dissolved in 20 mL *n*-hexane with addition 5 grams of silica gel. The mixture was stirred until homogeneous at 300 rpm for 1 hour. The samples that had been completely mixed in silica gel were evaporated at 40°C in order to remove *n*-hexane and wrapped in whatmann paper and put in the extraction thimble. Crude non-polar extract was isolated by using chloroform 350 mL at a maximum temperature of 50°C, then collected and evaporated to remove the solvent.[6]

Analysis of phytosterol levels
The isolate (0.001 g) was dissolved in *n*-hexane and put into a 10 mL tin flask. It was made sufficient with *n*-hexane to the marking margin then homogenized. Then 1 µL was injected into the GC.[9]

Identification of Isolate purity
The identification of isolate purity was performed using FT-IR and GC-MS by comparing the isolate spectrum with the standard and literature standards.[4, 10]

RESULTS AND DISCUSSION

Palm fatty acid distillate (PFAD) used in this study was brownish-yellow and solid-shaped at room temperature with the following characteristics: the saponification number was 177.58 mg KOH/g and the amount of free fatty acid was 89.905% as described in table 1 below.[5]

Table 1: Composition of fatty acid in the sample.

Names of Components	Names of Compounds	% Area
C: 12–0	Lauric Acid	0.4154
C: 14–0	Mirror Acid	0.7127
C: 16–0	Palmitic Acid	44.8977
C: 18–0	Stearic Acid	3.6305
C: 18–1	Oleic Acid	42.0810
C: 18–2	Linoleic Acid	6.1118
C: 18–3	Linolenic Acid	0.0666

Based on the table 1. above, it can be seen that the major fatty acids content present in the palm oil fatty acid distillate (PFAD) are palmitic acid (44.8977%) and oleic acid (42.0810%).

Saponification results obtained non-polar crude extract (unsabbed portion) with a concentration of 2,683 ppm. This number is quite large if we compare with previous research.[14]

Sokhlet isolation technique is a technique commonly used for the extraction of solid/hard compounds. However, the sample in this study was a non-polar crude extract of PFAD saponification in liquid form. To solve this problem, modification of the sokhlet was done by using a fat-free silica gel binder. The solvent used to isolate phytosterols was *n*-hexane. The isolation process lasted for 3 hours at a maximum temperature of 50°C with the first sample soaked with solvent for 1 night. After 3 hours the entire isolation result was collected and evaporated to remove the solvent. The weight of the phytosterol isolates obtained was 1.25 g of 5.5 g total crude non-polar extract.

The phytosterol isolate obtained then was analyzed using GC. The Figure chromatogram analysis of phosphate saposterol isolates can be seen in Figure 1 below.

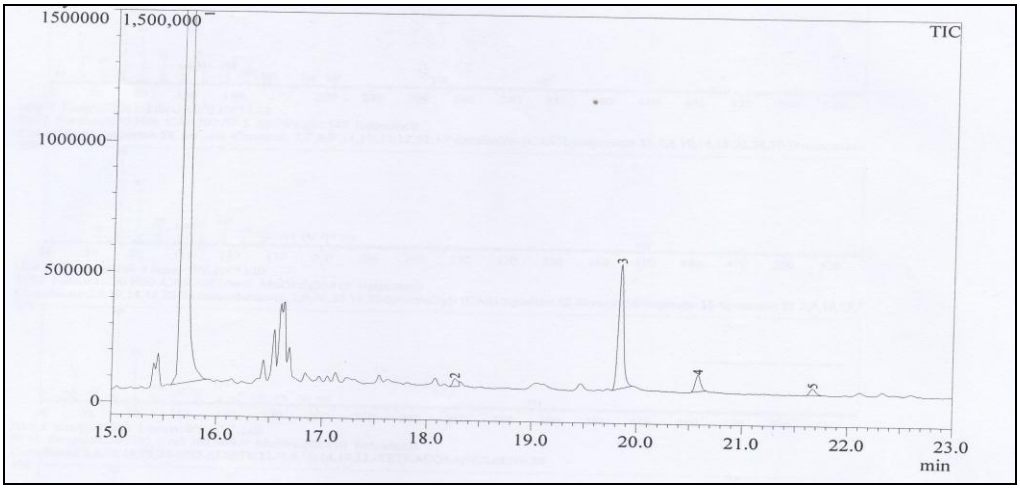

Fig. 1: Chromatogram of phytosterol isolate.

The chromatogram above showed that there were still impurities contained in the isolate suspected was fatty acid that left behind and has not been stabbed in the process of saponification. The amount of phytosterol isolate after isolation was 4,035 ppm. This fact of course increased almost 2-fold compared with phytosterol levels in the results of non-polar analysis of saponification results.

The identification of phytosterol compounds using FT-IR was aimed to look at the similarity of spectrum isolates with standard and to see specific functional groups owned by isolates corresponding to the literature. We can see the general structure of phytosterol in Figure 2 below and comparison of IR spectrum phosphosterol isolates with standard as represented in Figure 3.

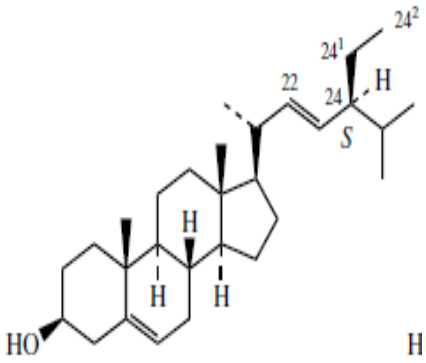

Fig. 2: Stigmasterol structure.

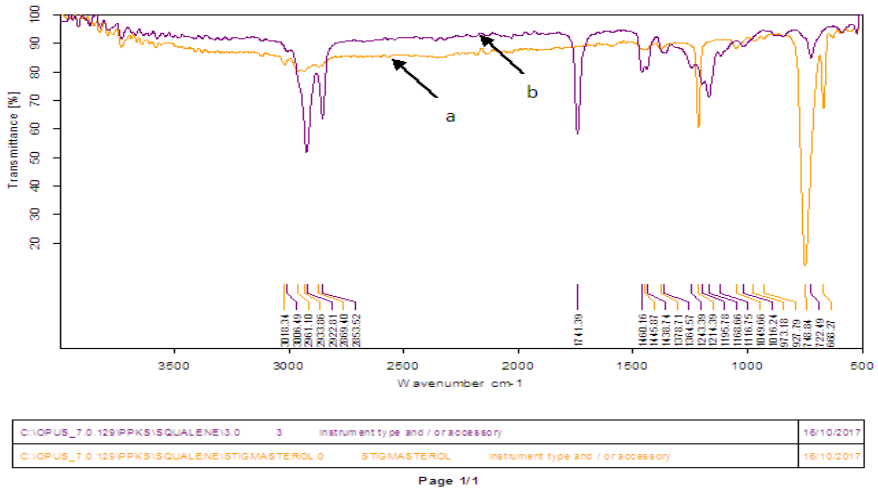

Fig. 3: Comparison of IR spectrum.

Based on the IR spectrum figure above, it can be seen that the isolate and standard squalene have functional groups as shown in table 2 below:

Table 2: Comparison of functional groups arising from isolate and phytosterol standard

Wave Numbers (cm^{-1})		Requitements	Vibration
Isolate Phytosterol	Standard Phytosterol		
3006	3018	3000-3040	C=C
722	748 ; 927 ; 973 ; 1049	720-1379	C-C
2853 1438, 1460	2869 1440	2720-2870 1428-1460	CH_2
2932 1364 ; 1438 ; 1460	2933 ; 2961 ; 1378 ; 1445	2900-2970 1254-1470	CH_3
1741	-	1690-1760	C=O
1016 ; 1116 ; 1243	1214	1080-1300	C-O

As represented in table 2, it can be seen that phytosterol isolates with standard phytosterols almost have the same wave numbers, therefore both of them have the same functional groups. But then in isolates, peak appeared at wave number 1741 cm^{-1} indicating the functional carbonyl group C=O, somehow this was suspected to be derived from fatty acids remained in the isolates due to the imperfect saponification process.

The identification of phytosterol compounds using GC-MS was aimed to look at fragments of isolates and compared them with libraries. The compatibility of isolates with the libraries indicated the level of compound purity. For more infomation, Figure 4 below shows the spectrum of phytosterol isolates using GC-MS.

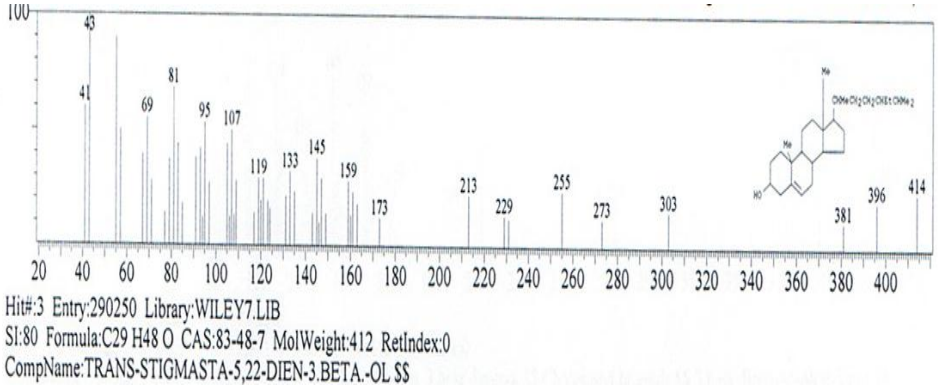

Fig. 4: Spectrum of MS phytosterol isolate.

The figure of MS phytosterol isolate exactly showed that phytosterol isolate has similarity with standard based on literature up to 80%.

CONCLUSION

Based on the result of the research, it can be concluded that the squalene compound in the palm fatty acid distillate (PFAD) can be isolated by using soxhlet modified with silica gel as binder. The isolation process begins with saponification in order to improve the isolate purity and reduce impurities (FFA) which somehow can be disruptive during the analysis process and isolation of the compound. The phytosterol isolate obtained in this study was 1.25 g with a concentration of 4,035 ppm. Identification of isolate with FT-IR showed a similarity of functional groups between IR spectra of isolate with standard, but there were impurities showing the functional carbonyl group C=O at wave number 1741 cm^{-1}. Meanwhile, the dentification using GC-MS showed the level of purity of phytosterol isolate based on the literatures reaching up to 80%.

The advantages of this method compared with Column Chromatography (CC) and Preparative TLC are the least of the solvents used, the size of the sample per batch unit and the shorter in time operation.

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Conflicts of Interests

There are no conflicts of interest.

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