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Properties of Ethanolysis Product from Ketapang Seed Oil (*Terminalia Catappa* Linn) Incorporated in Mucoadhesive Patch Film

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Abstract . The propose of these research was to prepare monoacilglyserol (Ethanolysis Product from Ketapang Seed Oil/EPKSO) by ethanolysis reaction and to measured it's antibacterial activity as free materials and as immobilizes material in starch film/patch. The oil of ketapang seed was extracted by pressing method. Fatty acid in the oil was identified with Gas Chromatography (GC). Ethanolysis process used 5 mL ketapang seed oil and 2mL NaOH in 100 mL ethanol 95%. Sample EPKSO from ethanolysis of ketapang seed oil were compare to EPKSO standard from SEAFAST Centre. The preparative TLC of ethanolysis product was eluted with n-hexane: diethyleter: formic acid (80:20:2) v/v. EPKSO was produced by TLC preparative from ethanolysis product. Antibacterial activity of EPKSO from ethanolysis reaction was assayed against Streptococcus mutans with diffusion method. From this research, ketapang seed oil obtained was 7.69 % w/w. The major fatty acid from ketapang seed oil from Gas Chromatography (GC) were palmitic acid, stearic acid, oleic acid and linoleic acid. The minor of fatty acid were miristic acid, palmitoleic acid, heptadecanoic acid, cis-10-heptadecanoic acid, belaidic acid, arachidonic acid, cis-11-eicosenoic acid, linoleic acid, cis-11.14-eicosedienoic acid, behenic acid, trisanoic acid and lignoseric acid. Both of the EPKSO have similar Rf (0.07). The amount of EPKSO result from ethanolysis ketapang seed oil was 1.79 % w/w. This EPKSO can inhibit S. mutans at 0.4 % w/v with inhibition zone 183 mm2. EPKSO was incorporated in starch based film/mucoadhesive patch with variation 2 MIC, 4 MIC 6 MIC dan 8 MIC. After incorporated with EPKSO starch based film/mucoadhesive patch can inhibit the growth of Streptococcus mutans at all EPKSO concentration in film/patch. Most of mechanical properties of film/patch still meet the range of standard. Organoleptic assay showed no significant difference between starch film with and without EPKSO.

1. Introduction

Ethanolysis Product from Ketapang seed oil (EPKSO) could play rule as the major type of food emulsifiers used in many food systems and are also important as a basic starting material to prepare several other derivatives of modified functional properties [1]. In particular, similar molecule like EPKSO have been used as surface-active agents in many industrial cleaning products such as detergents, shampoos, lotions, and tooth pastes or as raw materials for the synthesis of chemical compounds such as alkyd resins [2].

The two most prevalent commercial preparations of mono-and diacilglycerols are (1) Direct esterification (ethanolysis) of glycerol with a fatty acid, and (2) Glycerolysis of natural or hydrogenated fats or oils. The glycerolysis procedure is more economical because fats are cheaper than fatty acids and less glycerol is required. Fats and fatty acids are insoluble in glycerol and, in the absence of solvent; elevated temperatures are required to force the reaction to proceed. Direct esterification may be

catalyzed either by acids or bases. The ratio of glycerol to fatty acid determines the concentrations of mono-, di- and triacylglycerols in the final product [3]. Ketapang (*Terminalia catappa*) one of many vegetable oil in Indonesia. Ketapang seed oil consist of 58.66% (v/w) oil. The composition of this oil are palmitic acid 0.38%, palmitic oleic acid 35.26%, stearic acid 4.55%, oleic acid 38.72% and linoleic acid 20.57% [4]. According to those research, ketapang oil has potential to convert to be monoacilglyserol.

2. Materials and Methods

2.1. Preparation and Extraction of Ketapang Seed Oil

Sample of the ketapang seed was dark red and dry. It was taken from Sriwijaya University area in Indralaya. Ogan Ilir, South Sumatra. The sample of the ketapang seed was cleansed from skin and waste. Ketapang seed then was heated in the temperature 80°C in the oven through to constant weight and afterwards the sample was eroded. After that the sample was ready to be extracted. Ketapang seed that was eroded then was put into the implement press with the pressure 100 kg/cm^2 for 10 minutes. The ketapang seed oil was kept in the close place [5].

2.2. Analysis Composition of Ketapang Seed Oil with Gas Chromatography (GC)

Hence, concentration profiles of ethyl oleic and ethyl linoleic may be regarded as representative of and proportional to overall FAME (fatty acid methyl ester), with a proportionality constant of 0.7, equal to the combined mole fractions of triolein and trilinolein in the oil. A Shimadzu GC-17A with a liquid autosampler–autoinjector (held at 380°C) and FID detector (400°C) was used to obtain transient concentration measurements of external standard calibrations. The GC was fitted with a Restek MXT Biodiesel TG column (15 m 0.32 mm ID 10 l m) and employed both a temperature program (50–380 C) and a pressure program (20–65 kPa) [6].

2.3. Ethanolysis of Ketapang Seed Oil

This reaction is carried out according to molar ratio that optimizes the production of Ethanolysis Product from Ketapang Seed Oil. The molar ratio is Ketapang seed oil in: NaOH 2% ethanol 95% was 1:1 (v/b) which was the total weight of the two substrates is of 100 g. The reaction was carried out with agitation under 200 rpm at room temperature for 6 minute. Reaction was stopped with 0.2 mL chloride acid 6 N. Unreacted ethanol was separated and washed with distilled water. Water was released with sodium sulfate anhydrate. Ethanolysis products were separated with TLC silica gel G 60 F 245 to get Ethanolysis Product from Ketapang Seed Oil. The sample in TLC were eluted with mix of hexane/diethylether/formic acid 80:20:2 (v:v:v). The separated spots from TLC were lighten up with iodium vapor and diluted with diethylether. The extract then was heated at 100°C to get Ethanolysis Product from Ketapang Seed Oil [7].

2.4. Antibacterial Assay of Ethanolysis product from Ketapang Seed Oil

The antibacterial activities of the ethanolysis Product from Ketapang Seed Oil were analyzed by agar well diffusion method. The Ethanolysis Product from Ketapang Seed Oil was tested against the selected bacterial strains. The plate were washed and placed in an autoclave for sterilization. After sterilization, nutrient agar medium was poured into each sterile plate and allowed to solidify in a laminar air flow chamber. After solidification, using a sterile cotton swabs, fresh bacterial culture with known population count was spread over the plate by spread plate technique. Then one well of 5mm size made in the agar plates with the help of sterile cork borer, the wells were loaded with 60µl of Ethanolysis Product from Ketapang seed oil standard (0.2%) and Ethanolysis Product from Ketapang Seed Oil (0.2%). All the plates were incubated at 37℃ for 24-48 hours. After incubation, the plates were observed for formation of clear inhibition zone [8].

2.5. Incorporation Ethanolysis Product of Ketapang Seed Oil In Starch based film/Patch

Edible film was made by mixing 5 grams sweet sago starch, 1 ml glycerol, carboxymethilcellulose with various concentration and 100ml of distilled water. All mixed and heated (temperature 65-70°C) until

the dough gelatinized. After the mixture was cooled to a temperature of 40°C, ethanolysis of ketapang seed oil was added. The mixture then spread on a mold, and dried. After the starch based film dried, it can be taken from the mold.

2.6. Antimicrobial activity of films

Antimicrobial activity test on films/patch was carried out using the agar diffusion method according [6]. The inhibition zone of the assay on solid media was used to determinate the antimicrobial effects of films against *Streptococcus mutans*. The edible films were cut into 6-mm-diameter disks and then placed on Nutrient agar (Merk, Darmstadt, Germany) plates, which had been previously seeded with 0.2 mL of inoculums containing approximately 105 to 106 CFU/mL of *Streptococcus mutans*. The plates were then incubated at 37°C for 24 h. After that, the plates were examined for "zone of inhibition" of the film discs. The contact area was used to evaluate growth inhibition underneath the film disk in direct contact with target microorganisms in the agar. The area of the whole zone was calculated and then subtracted from the film disk area, and this difference area was reported as zone of inhibition [14].

2.7. Physicochemical Properties of Films

The thickness value represented by the mean of five measurements taken along the strips made on each film which used for testing tensile strength and percentage elongation at break. The films thickness measured automatically by a micrometer connected to the Universal Testing Instrument (Zwick Z010). Water vapour permeability (WVP). Carried out by using a modified method to that described by [12]. Fan was provided for the air circulation inside the desiccator cabinets at the first for 4 hours only after that, the test completed without it. All the tests were performed in triplicates. Tensile strength (TS) and percent elongation (%E). Tensile testing was performed with the Universal Testing Instrument (Zwick Z010) on (50 ×4 cm) dample shape film strips. Initial grip separation set at 25 mm, while cross-head speed set at 50 mm/sec, the used lot cells (100 N) [13].

3. Results and Discussion

3.1. Analysis of Fatty Acid From Ketapang Seed Oil with Gas Chromatography

The Figure 1 showed the existence 22 peaks that identified the existence 22 components. The peak with retention time less than 10 minutes came from solvent, whereas other peaks were the fatty acid. Table 1 showed that there were 16 fatty acid of ketapang seed oil. Table 1 showed there were saturated fatty acid such as miristic acid, palmitic acid, heptadecanoic acid, stearic acid, arachidonic acid, behenoic acid, tricosanoic acid and lignoseric acid. Fatty acid from ketapang seed oil can be seen in Table 1.

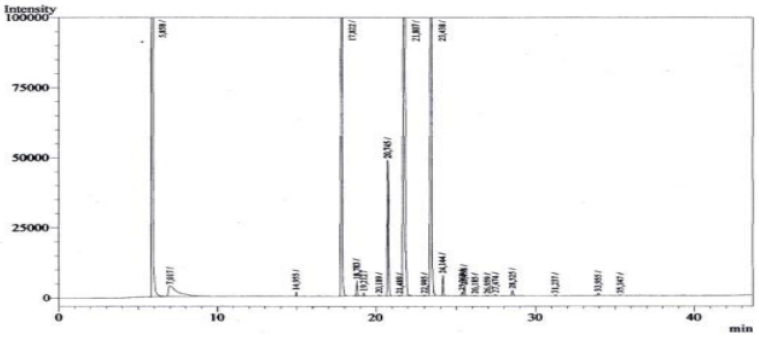

Figure 1.Chromatogram of Fatty Acid from Ketapang Seed Oil

Table 1. Fatty Acid from Ketapang Seed Oil

No.	Fatty acid	Retention Time (minute)	Concentration (% w/w)
1.	Myristicacid	14.955	0.09
2.	Palmitic acid	17.822	19.50
3.	Palmitoleic acid	18.783	0.36
4.	Heptadecanoic Acid	19.212	0.07
5.	Cis-10-heptadcanoic acid	20.189	0.03
6.	Stearic acid	20.745	3.94
7.	Elaidic acid,	21.489	0.03
8.	Oleic acid	21.807	30.35
9.	Linoleic acid	23.438	27.87
10.	Linolenic acid	25.498	0.30
11.	Arachidonic acid	24.144	0.51
12.	Cis-11-Eicosanoic acid	25.369	0.01
13.	Cis-11,14 Eicosadienoic acid	27.474	0.05
14.	Behenoic acid	28.525	0.16
15.	Trikosanoic acid	31.237	0.02
16.	Lignoseric acid	33.955	0.07

The unsaturated fatty acid that exist in the oil were palmitoleic acid, cis-10- heptadecanoic acid, elaidic acid, oleic acid, linoleic acid, cis-11-eikosanoat acid, linolenic acid and cis-11,14- eikosadienoic--acid.

3.2. Ethanolysis of Ketapang Seed Oil and Preparation of Ethanolysis Product From Ketapang Seed Oil

The results of the extraction was 250 mL ketapang seed oil. Ethanolysis was done for 10 mL oil and gave 8,07 gram of the product. To make sure that the product of ethanolysis was difference with the oil, the two ingredient then were compared by using TLC (Figure 2).

Figure 2. Chromatogram of TLC Ethanolysis Product (Ethanolysis Product from Ketapang Seed Oil) (I) and (II) Ketapang Seed Oil

According to Figure 2, there were the differences spot between ethanolysis product and the ketapang seed oil. To know whether ethanolysis product from Ketapang Seed Oil was being formed by ethanolysis reaction, TLC was used to compared with Ethanolysis Product From Ketapang Seed Oil

standard from South East Asian Food and Agricultural Science and Technology Center (SEAFAST Center) IPB. Ethanolysis Product from Ketapang Seed Oil standard from SEAFAST centre came from palm oil that had purity 95% and 5% still exist of mixture DAG and TAG. Stationary phase that had been used was gel silica 60 GF254 and the mobile phase with three eluent that was different composition, (1).n-hexane: diethyl eter: acid format (80:20:2 v/v), (2). ether petroleum: diethyleter: acetic acid glacial (70:30:0,2v/v), and (3). n-hexane: format acid (80:2 v/v) [8]. Iodium vapor was used to light the spot. From the TLC showed that Ethanolysis Product From Ketapang Seed Oil from ethanolysis reaction and Ethanolysis Product From Ketapang Seed Oil from SEAVAST centre has the same Rf (0.06).The same Rf refer to the same highest fatty acid content in both sample that was oleic acid. From the Figure 3, the separation was good with composition mobile phase were n-hexane: diethyl eter: format acid (80:20:2) v/v. TLC spot of ethanolysis product were given in Figure 3.

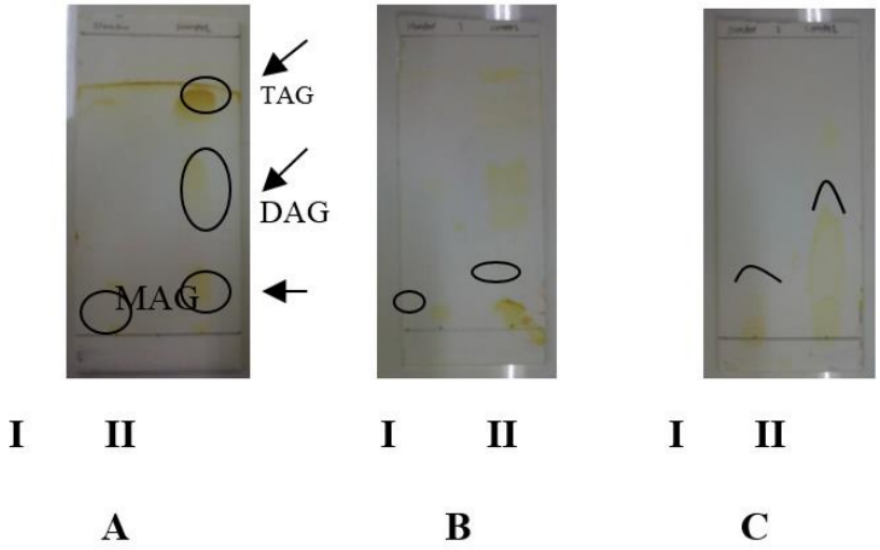

Figure 3. TLC of Ethanolysis Ketapang Seed Oil and Ethanolysis Product From Ketapang Seed Oil standard with n-hexane; diethyleter, formic acid (80:20:2 v/v) A, petroleum ether: diethyleter : acetic glacilic acid (70:30:0.2 v/v) B.and n-hexane: formic acid (80:2 v/v) C, I= Ethanolysis Product From Ketapang Seed Oil standard (from SEAFAST IPB), II= Ketapang Seed Oil

From 8.07 gram product of ethanolysis, 2.76 gram was taken for further purification with TLC preparative to Ethanolysis Product from Ketapang Seed Oil. Silicagel 60 GF254 was used as quit phase and the solvent n-hexane: diethyl eter: format acid (80:20:2) v/v was used as movement phase, because gave good separation from earlier research.

3.3. Antibacterial Assay of Standard and Ethanolysis Product from Ketapang Seed Oil against S. mutans

Based on the Figure 4, inhibition zone of Ethanolysis Product from Ketapang Seed Oil against S. mutans smaller compared with Ethanolysis Product from palm Oil standard From Seavast Centre. Ethanolysis Product from Ketapang Seed Oil standard had inhibitor zone 160 mm2, whereas Ethanolysis Product from Ketapang Seed Oil had inhibition zone only 83 mm2.The difference of inhibition from Ethanolysis Product from Ketapang Seed Oil standard and Ethanolysis Product from Ketapang Seed Oil towards the growth of the *S. mutams* colony was suspected by the differences of the compound

content in each Ethanolysis Product from Ketapang Seed Oil that responsible to decline the growth of bacteria [9]. The inhibition zone of ethanolysis product can be in Table 2..

Table 2. Antibacterial Activity of Ethanolysis Product from Ketapang Seed Oil Against S. mutans

Sample	Concentration	Inhibition Zone (mm^2)
Ketapang Seed Oil	0.5%	0 ± 0.0
Ethanolysis Product from Ketapang Seed Oil	0.5%	183 ± 2,1
Standard from SEAVAST	0.5%	160 ± 3.4

Product standard (from SEAFAST Centre) was made from palm oil whereas EPKSO sample came from ethanolysis of ketapang seed oil. Both this oil had the different fatty acid composition. The difference composition and the fatty acid from the two oils caused the differences of antibacterial characteristic [10]. The well diffusion method of ketapang seed oil and it's ethanolysis product can be seen in Figure 4.

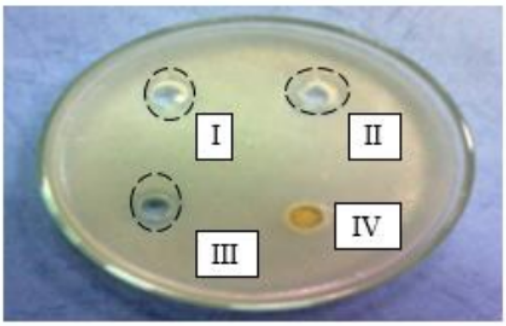

Figure 4. The inhibition zone Ketapang seed oil: EPKSO sample (I); MAG standard (II), Solvent (III), Ketapang Seed oil (IV)

3.4. Antibacterial of Starch based film/Patch Containing Ethanolysis Product from Ketapang Seed Oil (EPKSO)

Inhibitory activity was measured based on the clear zone surrounding a circular film disk. If there is no clear zone, it is assumed that there is no inhibition. The results showed that the films containing ethanolysis product of ketapang seed oil (EPKSO) were effective against S. mutans. As the concentration of EPKSO increased, the zone of inhibition also increased significantly ($P < 0.05$). The results of the antimicrobial assessment of starch based film/Patch with incorporated EPKSO against S. mutans are presented in Table 3.

The presence of glycerol as plasticized in the film/patch markedly affects the inhibitory effect of the EPKSO in the film. The results showed that EPKSO exhibited significantly ($P < 0.05$) higher antimicrobial activity in the presence of glycerol, as evidenced by larger inhibitory zone at all EPKSO concentrations. This could be attributed to the increased solubility of EPKSO in the matrix and more uniform dispersion of the oil in the film. However, there was no significant difference ($P > 0.05$) in the inhibitory zone for plasticized and unplasticized films /patch without EPKSO.

Colour of the film increase become clear, the value clearness increase from 62 become 67.1%. Tensile strength increase from 2.71 become 1.51 Kgf/cm , elongation increase from 68.95 to 185.3%.

From Table 4 respondent gave positive response on colour for film incorporated with EPKSO. Perception value of respondent increase from 3.4 become 3.9. Respondent perception on aroma also increase from 4.1 become 4.4 . Respondent gave neutral perception for the taste of film(patch) the value was 3.83 for unincorporated film become 3.79 <0.05) for incorporated film/patch.

Table 3. Antibacterial Activity of EPKSO in Starch Based Film/Patch Against S. *mutans*

Starch Film/Patch	Concentration of EPKSO	Inhibition Zone (mm^2)
Starch Based Film/Patch with no glycerol	0.0%	0 ± 0,0
Starch Based Film/Patch with glycerol	0.0%	0 ± 0,0
Starch Film/Patch Incorpoated with EPKSO and without glycerol	2 MIC	190 ± 1,1
Starch Film/Patch Incorpoated with EPKSO and with glycerol	4 MIC	201± 1,8
Starch Film/Patch Incorpoated with EPKSO and without glycerol	6 MIC	258 ± 1,2
Starch Film/Patch Incorpoated with EPKSO and with glycerol	8 MIC	283 ± 2,2

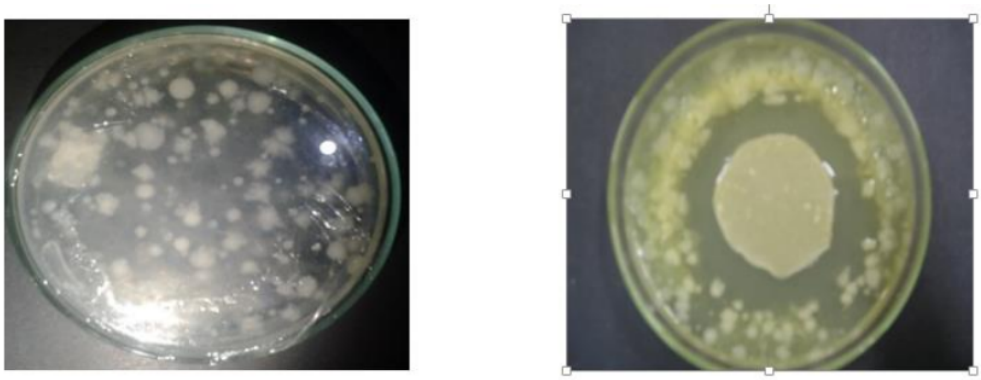

Figure 5. Inhibition Zone of Edible Film Containing Ethanolysis of Ketapang Seed Oil against *Streptococcus mutans*

3.5. Characteristic Starch film/Patch Properties Before and After Incorporated with EPKSO
Physical properties of starch film/patch can be seen in Table 3. There are significantly difference between film/patch without and with EPKSO (P<0,05). aw of starch film/patch before incorporating with EPKSO was 0.57 and become 0.36, after incorporating with EPKSO. This aw value can inhibit the growth of bacteria. The thickness, elongation and Oxygen transmission were increase significantly. The thickness have range between 0.13 mm-0.16 mm, O2 transmission increase from 38.25 become 51.61 cm2/m2 24 h. Transmission of H_2O decrease from 45.93 become 25.33 g/m^2. 24 h.

Table 4. Properties of starch film /patch before and after incorporation with EPKSO

No.	Characteristic of film edibel	Sample Starch Film/Patch	
		Without EPKSO	Sago Starch+EPKSO
1.	a_w	0.57±0.2	0.36±0.1
2.	Colour (%)	62.80±1.1	67.91±1.2
3.	Thickness of film (mm)	0.13±0.3	0.16±0.5
4.	Tensile Strenght (kgf/cm)	2.71±1.1	1.51±1.3
5.	Elongation(%)	68.95±1.6	185.3±2.1
6.	Transmission of O_2 (cm^2/m^2.24h)	38.25±1.8	51.61±1.9
7.	Transmission of H_2O (g/m^2/24 h)	45.93±2.2	25.27±3.3
Organoleptic Assay		Score from respondent*	
	Colour	3.4±1.4	3.9±1.9
	Taste	3.83±1.7	3.79±1.1
	Aroma	4.1±1.2	4.4±1.5

*)1=No interested at all; 2=No interested; 3=plain; 4= interested; 5=very interested

Table 5. Characterization film (patc) from sago starch.

No	Characteristic of Starch based Film/Patch	Starch film/Patch Incorported with EPKSO	Standard Value (Grade)
1.	a_w	0.36±0.1	-
2.	Clearness (%)	62.91±1.2	-
3.	Thickness (mm)	0.14±0.5	Max 0.25
4.	Strengsile strengt (Kgf/cm)	1.51±1.3	Min 1.0
5.	Elongation of percentage (%)	185.3±2.1	Min 50
6.	Laju transmisi gas O_2 (mL/m^2 h)	51.61±1.9	Max 50
7.	Transmission of vapor (g/m^2.24 h)	25.27±3.3	Max 50

3.6. Comparation Characteristic of Film With Standard

Comparation between incorporated film/patch with standard (Japanese Industrial Standard, 1975). The value can be seen in Table 5. According to JIS standard mentioned by [11], the film exist in range of standard 7-13. Water activity (aw) sago starch film/patch which was incorporated with KPSO showed height value from sago starch which was incorporated EPKSO. More decrease aw film/patch can preserve from degradation by bacteria. Film which was incorporated with EPKSO, has 5 percent more clear from film/patch which was not incorporated with EPKSO

4. Conclusions

Ketapang seed oil consisted of saturated fatty acid which include meristic acid (C14:0), palmitic acid (C16:0), hepta decanoic acid (C17:0), stearic acid (C18:0), arachidonic acid (C20:0), behenic acid (C20:0), tricosanoic acid (C23:0) and lignoseric acid (C24:0), and the unsaturated fatty acid which include palmitoleic acid (C16:1), cis-10- heptadecanoic acid (C17:1), elaidic acid (C18:1n9t), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), cis-11- eicosanoic acid (C20:1), linoleic acid (C18:3n3) and cis-11,14- eikosadienoic acid (C20:2). From this research the amount of Ethanolysis product from ketapang seed oil was 0,036 gram and equal to 1.2 % w/w from all ethanolysis oil results. Ethanolysis product

from ketapang seed oil can inhibit the growth of the Streptococcus mutans in the concentration 0.5 of % w/v. Incorporated EPKSO in starch film/patch can turn the film/patch to be antibacterial film, with still had good mechanical characteritic compare to standard value. Organoleptic assays showed no significant differences ($P<0.05$) between starch film/Patch with and without EPKSO.

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