

Toxicity Testing of White Kapul Fruit Rind Extract (*Baccaurea macrocarpa*) and Component Analysis using Chromatography Method

Uji Toksisitas Ekstrak Kulit Buah Kapul Putih (*Baccaurea macrocarpa*) dan Analisis Kandungan Senyawa dengan Metode Kromatografi

Kholifatu Rosyidah^{1,*}, Lida Karmila¹, Maria Dewi Astuti¹

¹Chemistry Study Program, Faculty of Mathematics and Natural Sciences,
Lambung Mangkurat University, Banjarbaru, Indonesia

Email: krosyidah@ulm.ac.id

ABSTRACT

White Kapul was used in this study. This study aimed to determine the toxicity of the white Kapul fruit rind extract and its chemical content analysis using TLC and GC-MS. The extraction method used was gradual maceration, namely technical n-hexane, technical ethyl acetate, and technical methanol. Toxicity test using the BSLT method with *Artemia salina* larvae at the nauplii stage. The test showed that the LC₅₀ value of ethyl acetate extract, methanol extract, and n-hexane extract was 350.87 ppm, 485.61 ppm, and 735.932 ppm, respectively. Based on LC₅₀ values, all extracts had the potential as pesticides. The third extract of white Kapul fruit rind was carried out by TLC analysis to determine the pattern of its compound content. The results of the TLC analysis showed that each extract had different polarity compounds according to the polarity of each solvent. The most active extract, ethyl acetate extract, was further analyzed using GC-MS. There were 32 peak compounds at a retention time of 25.384 to 65.725 minutes in GC-MS analysis. Four compounds with the largest percentage area were gynoloton (58.09%), 15-chloro-4-pentadesene (16.25%), 17- (acetyloxy) -2-methyl-, (2 α .,5 α ,17 β) - estra-3-on (6.07%), and methyl-11-octadesenoate (5.98%).

Keywords: *Baccaurea macrocarpa*, Euphorbiaceae, TLC, toxicity, BSLT, GC-MS

ABSTRAK

Buah kapul putih digunakan pada penelitian ini dengan tujuan mengetahui toksisitas ekstrak kulit buah kapul putih dan analisis kandungan kimianya menggunakan KLT dan GC-MS. Metode ekstraksi yang digunakan adalah maserasi bertahap dengan pelarut n-heksana teknis, etil asetat teknis, dan metanol teknis. Uji toksisitas menggunakan metode BSLT dengan larva *Artemia salina* tahap naupli. Uji toksisitas menunjukkan nilai LC₅₀ ekstrak etil asetat sebesar 350,87 ppm, ekstrak metanol sebesar 485,61 ppm, dan ekstrak n-heksana 735,932 ppm. Semua ekstrak bersifat toksik dan berdasarkan nilai LC₅₀ berpotensi sebagai pestisida. Ketiga ekstrak kulit buah kapul putih dilakukan analisis KLT untuk mengetahui pola kandungan senyawanya. Hasil dari analisis KLT menunjukkan bahwa tiap ekstrak memiliki senyawa yang berbeda kepolarannya sesuai kepolaran masing-masing pelarut. Ekstrak paling toksik yaitu ekstrak etil asetat selanjutnya dianalisis dengan GC-MS. Pada analisis GC-MS terdapat 32 puncak senyawa pada waktu retensi 25,384 sampai 65,725 menit. Empat senyawa dengan % area terbesar yaitu ginoloton (58,09 %), 15-kloro- 4-pentadesuna (16,25 %), 17-(asetiloksi)-2-metil-, (2 α .,5 α .,17 β .)-estra-3-on (6,07 %), dan metil- 11- oktadesenoat (5,98 %).

Kata Kunci: *Baccaurea macrocarpa*, Euphorbiaceae, KLT, toksisitas, BSLT, GC-MS.

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1. INTRODUCTION

The kapul fruit is a seasonal fruit that is indigenous to Kalimantan. Kapul fruit is not farmed and is typically harvested in the wild. According to Antarlina (2009), several Kalimantan fruits naturally grow in the neighboring yards, while some are forest plants (located in the forest). Fruit can be consumed as part of a healthy lifestyle to help overcome health problems such as nutritional and vitamin deficiencies (Susi, 2014). A fruit diet can also help boost the body's immunity during pandemic, as fruit is high in vitamins and antioxidant compounds. Astuti *et al.* (2020) discovered that Kapul fruit rind contained significant antioxidant activity. Kapul fruit rind was extracted in stages with n-hexane, ethyl acetate, and methanol as solvents. The highest IC50 value in methanol extract was 22.968 ppm, ethyl acetate was 29.741 ppm, and n-hexane was 141.931 ppm, while the IC50 value for vitamin C was 5.019 ppm as a comparison compound.

Several studies have been conducted on the bioactivity of the white Kapul fruit plant (*Baccaurea macrocarpa*). Yunus *et al.* (2014) stated that the extract and methanol fraction of the rind of Kapul fruit showed antibacterial activity against *E. coli* and *S. aureus* bacteria. The diameter of the inhibition zone of the methanolic extract of the capsule fruit peel against *S. aureus* bacteria at concentrations of 5%, 10%, and 20%, respectively, was 0.0: 4.33, and 6.40 mm; the n-hexane fraction was 3.55, 6.55, and 8.77 mm; the ethyl acetate fraction was 16.55, 19.05, and 22.01 mm; and the remaining methanol fraction was 6.92, 9.78, and 12.32 mm. The antibacterial activity against *E. coli* at concentrations of 5%, 10%, and 20% was 5.01, 7.82, and 9.21 mm; the n-hexane fraction was 8.22, 11.36, and 13.66 mm; the ethyl acetate fraction was 15.41, 20.25, and 23.92 mm; and the methanol fraction was 9.78, 13.43, and 15.02 mm.

Dwijayanti (2014) showed that the bark of the Kapul stem has toxicity activity.

Toxicity test was carried out using the BSLT method, and the LC50 value of the methanol extract was 318.150 ppm. The extract was then fractionated and tested for toxicity to obtain LC50 data for the ethyl acetate fraction of 310.443 ppm, the n-hexane fraction of 500.160 ppm, the methanol fraction of 602.869 ppm, and the chloroform fraction of 640.471 ppm.

Yunus *et al.* (2017) and Tirtana *et al.* (2013) stated that Kapul fruit contains secondary metabolites, including saponins, alkaloids, and flavonoids. The results of phytochemical tests by Dwijayanti *et al.* (2014) on Kapul bark extract and ethyl acetate fraction found the presence of alkaloids, steroids, flavonoids, and polyphenols. The methanol fraction contained flavonoid compounds, alkaloids, and polyphenols, while the chloroform and n-hexane fractions contained alkaloid compounds.

This study will examine the toxicity of the white Kapul fruit rind using *Artemia salina* Leach shrimp. The toxicity testing with the BSLT method is a simple bioassay method for exploring natural products. The advantages of this method are that it can be performed quickly, is reliable, and has been used for ±30 years in toxicological studies. The extraction method used was stepwise extraction with the different polarity of solvents such as n-hexane, ethyl acetate, and methanol.

1. MATERIALS AND METHODS

2.1. Materials

The materials used were white Kapul fruit rind, technical n-hexane, technical methanol, technical ethyl acetate, cerium sulfate, dimethyl sulfoxide (DMSO), sulfuric acid, aquadest, artificial seawater, and *A. salina* Leach. The tools used were rotary evaporator, GC-MS, spatula, O'haus analytical balance, glassware, distillation apparatus, TLC, petri dish, water bath, magnifying glass, *Artemia salina* Leach hatchery equipment, vials, blender, aerator,

glass stirrer, filter paper, drip plate, and dropper.

2.2. Sample preparation of white Kapul fruit rind

The rind of the white Kapul fruit was peeled and separated from the flesh. The rind was then cut into thin strips to facilitate the drying process. The drying process was carried out by air drying for 7 days. The dried rind was then ground with a blender until it became a powder, weighed, and stored in a container.

2.3. Extraction of white Kapul fruit rind

This research employed stepwise extraction, namely the maceration method with technical n-hexane, technical ethyl acetate, and technical methanol. A total of 500 g of dry powder of Kapul fruit rind was put into a maceration vessel, soaked in a non-polar solvent, namely n-hexane, in a ratio of 1:5 for 24 hours while stirring several times. The maceration was repeated 3 times. The maceration results were filtered with Whatman paper No. 1 and evaporated using a rotary evaporator at 40-50°C. Furthermore, the extract was concentrated with a waterbath to obtain a thick extract. The n-hexane maceration residue was extracted again using ethyl acetate as solvent. The residue from the ethyl acetate extraction was re-extracted using methanol. Each extract obtained was used for further testing (Anindya, 2012).

2.4. Toxicity test of white Kapul fruit rind extract

Shrimp larvae were put into artificial seawater equipped with an aerator for 1×24 hours. Artificial seawater was made from 20 grams of salt dissolved in 1 liter of distilled water. Toxicity test solution made from n-hexane, ethyl acetate, and methanol extract was weighed as much as 6.25 mg then dissolved in 25 mL of artificial seawater. Each extract was first dissolved using 0.5 mL of DMSO solution. Each extract was pipetted 1; 1.5; 2; 2.5; 3; 3.5; 4; 4.5; 5; and

5.5 mL into the vial and added artificial seawater until the volume reached 25 mL, so that the concentration of the solution became 100, 150, 200, 250, 300, 350, 400, 450, 500, and 550 ppm. The negative control solution contained artificial seawater without the addition of sample extract with 0.5 mL of DMSO. A total of 20 shrimp larvae were added to each vial containing the test and negative control solutions. After 24 hours, the number of dead shrimp larvae was observed, and the mortality percentage was calculated.

2.5. Thin layer chromatography analysis of white Kapul fruit rind extract

A total of 2 mg of each extract was weighed and dissolved with a few drops of solvent. The diluted extract was spotted on the TLC plate at 1 cm from the bottom line and the edge. The stationary phase was a silica gel plate 60GF254. The stationary phase was then eluted using the eluent with n-hexane and ethyl acetate in a ratio of 7:3 and 9:1, while the eluents of chloroform and methanol were in a ratio of 9:1. If the eluent reached the boundary line, the elution was stopped, and the stationary phase was further dried. The stationary phase was sprayed with cerium sulfate and analyzed using 254 and 366 nm UV lamps. The resulting stain was then calculated for its R_f value.

2.6. GC-MS analysis of white Kapul fruit rind extract

GC-MS analysis following Maharani *et al.* (2016). GC-MS using Shimadzu QP 2010 with column RTx-5MS, 30 m long, the temperature of the injector and detector was 250°C, and operating temperature of 50-300°C. The temperature at 50-120°C was set to 4°C/min and held for 1 minute. The temperature of 120-300°C with a temperature increase of 6°C/minute was held for 5 minutes with a total retention time of 60 minutes. The carrier gas was helium, with a molecular weight of 50-500.

A total of 10µL of the thick extract of the white Kapul fruit rind was dissolved in

240µL of solvent. 1µL of the solution was then injected into the GC-MS. The flow rate was set at 1 mL/min with a column temperature of 70°C held for 2 minutes and then increased to 37°C/min to 250°C and held for 10 minutes (Prakash *et al.*, 2001). Compound analysis was performed using the Wiley/NIST Library software.

2.7. Data analysis

The data obtained were in the form of *A. salina* shrimp larvae mortality in each treatment/extract concentration. The mortality percentage was calculated from the data on the number of dead larvae. Data on the percentage of larval mortality was used to find the probit value in the probit table. Next, a graph of the relationship between the concentration log (X-axis) and the probit value (Y-axis) was constructed to obtain a linear regression equation $y = a + bx$. The x value was converted into antilog form so that the LC₅₀ value was obtained.

2. RESULTS AND DISCUSSION

3.1. Sample preparation

The weight of fresh Kapul fruit was 6 kg. The Kapul fruit was first separated from the pulp, then the rind of the fruit was chopped into small pieces to speed up the drying process and dried for 7 days. Drying aimed to reduce the water content in the sample, making it less susceptible to contamination by microbes or fungi (Arwan, 2017).

The weight of dry Kapul fruit rind was 2.5 kg. The rind of the kapul fruit was grounded using a blender until it became a powder, then weighed and stored in a

container. After being mashed, the weight of the dried Kapul fruit skin was 2 kg. A total of 500 grams of dried Kapul fruit peel powder was then used for the maceration process.

3.2. Extract of white Kapul fruit rind

Gradual maceration was used to extract the rind of the Kapul fruit (*B. macrocarpa*). The solvent was changed daily throughout the maceration process to maximize the extraction of secondary metabolites from the rind.

Kapul fruit rind powder was soaked with a non-polar solvent, namely n-hexane, semipolar, namely ethyl acetate, and polar, namely methanol, for 3×24 hours gradually. The sample powder was soaked with n-hexane as a solvent to extract non-polar compounds. The dregs extracted with n-hexane were then soaked with ethyl acetate to extract semipolar compounds. The dregs from ethyl acetate extraction were then soaked with methanol to extract polar compounds.

The white Kapul fruit rind extract was then evaporated using a rotary evaporator. Evaporation is the process of separating the sample from the solvent. The temperature of the rotary evaporator was kept between 40 and 50°C to avoid decomposition of the compounds contained in the macerate owing to excessive heating. The evaporated extract was then concentrated with a waterbath. The yield of white Kapul fruit rind extract is presented in Table 1.

Table 1. The result of maceration extraction of white Kapul fruit rind

Solvent	Powder	Extract	Yield (%)
n-heksana		0.07 gram	0.014%-b/b
etil asetat	500 gram	1.20 gram	0.240%-b/b
metanol		13.57 gram	2.714%-b/b
Total		14.84 gram	2.968%-b/b

Based on Table 1, it is known that the highest yield was obtained from extraction with methanol. The yield stated the number of compounds in the Kapul fruit rind extract dissolved in methanol. Kapul fruit rind has different yield values, indicating that polar secondary metabolites' content is high. It means that the methanol extract has a higher yield value compared to the ethyl acetate and n-hexane extracts. The total yield obtained was 2.968%. The difference in yield resulting from the gradual extraction is higher than Dwijayanti (2014). It may occur because the extraction was carried out with methanol solvent first and then continued with other fractions. Dwijayanti (2014) extracted Kapul bark with methanol as a solvent. Extraction of 3.3 kg of Kapul bark with methanol as a solvent resulted in 58.28 grams of methanol extract and 1.76% yield. Thus, the rind of the Kapul fruit contains a higher concentration of secondary metabolites than the bark.

3.3. Toxicity Test of White Kapul Fruit Rind Extract

The toxicity test was carried out with three variations: n-hexane extract, ethyl acetate, and methanol. The solubility of extracts in artificial seawater often causes problems because of the different levels of polarity. Extracts are difficult to dissolve in artificial seawater, so Dimethyl sulfoxide (DMSO) needs to be added. In general, DMSO is used as a surfactant to dissolve extracts in artificial seawater. A total of 0.5 mL of DMSO was added to 25 mL of mother liquor for each extract. The results of the toxicity test of the white Kapul fruit peel extract is presented in Table 2.

Meyer (1982) stated that the extract is toxic to the BSLT test if it resulted in the death of 50% of the test animals at a concentration of $LC_{50} < 1000$ ppm, which indicates that the sample has potential as an anticancer, antibacterial, anti-fungal, pesticides and so on. Rizqillah (2013) stated that compounds that have the potential as anticancer are found at an LC_{50} value of 0-30 ppm, compounds that have the potential as an antibacterial is at an LC_{50} of 30-200 ppm, and compounds that have the potential as pesticides are at an LC_{50} value of 200-1000 ppm.

Table 2. Toxicity test results of white Kapul fruit rind extract

Extract	Concentration (ppm)	% Mortality	Concentration Log	Probit	LC_{50} (ppm)
n-heksana	100	10.0	2.00	3.72	735.932 ppm
	200	17.5	2.30	4.07	
	300	25.0	2.48	4.33	
	400	30.0	2.60	4.48	
	500	41.3	2.70	4.78	
Etil Asetat	100	10.0	2.00	3.72	350.87 ppm
	150	17.5	2.18	4.07	
	200	25.0	2.30	4.33	
	250	35.0	2.40	4.61	
	300	43.8	2.48	4.84	
Methanol	350	16.3	2.54	4.02	485.61 ppm
	400	25.0	2.60	4.33	
	450	30.0	2.65	4.48	
	500	56.3	2.70	5.16	
	550	71.3	2.74	5.56	
Control	0	0	-	-	-

*Note: - = No value

The extract with the highest toxicity was ethyl acetate extract with an LC_{50} value of 350.87 ppm, methanol extract of 485.61 ppm, and n-hexane extract with an LC_{50} value of 735.932 ppm. Dwijayanti (2014) stated that the bark of the Kapul fruit has the toxicity power of methanol extract with an LC_{50} value of 318.150 ppm. While the

ethyl acetate fraction with an LC_{50} of 310.443 ppm, the n-hexane fraction of 500.160 ppm, the remaining methanol fraction of 602.869 ppm, and the chloroform fraction of 640.471 ppm. The toxicity test showed that Kapul fruit rind and bark extract has potential pesticides.

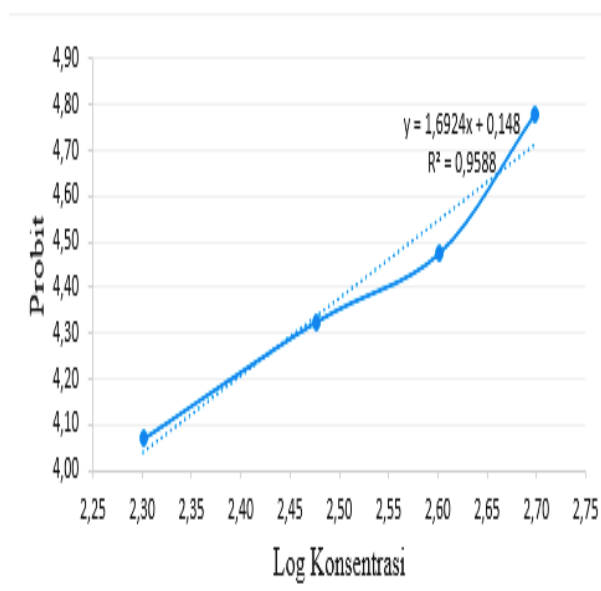


Figure 1. The Relationship Graph between Concentration and Probit of n-hexane of Kapul Fruit Rind Extract

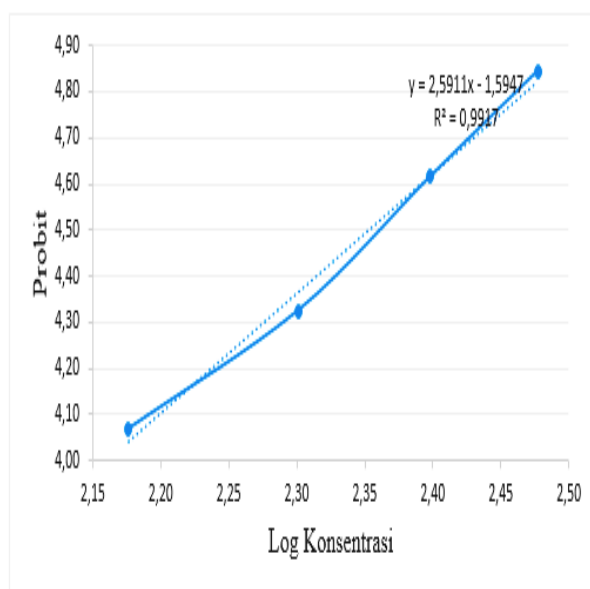


Figure 2. The Relationship Graph between Concentration and Probit of Ethyl Acetate Extract of Kapul Fruit Rind

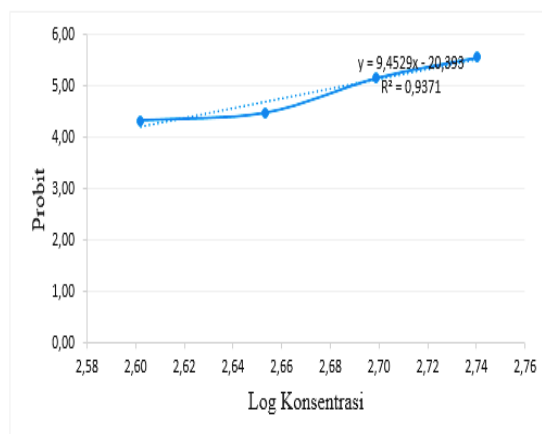


Figure 3. The Relationship Graph between Concentration and Probit of Methanol Extract of Kapul Fruit Rind

3.3. Thin layer chromatography analysis of white Kapul fruit rind extract

TLC analysis was carried out on the Kapul fruit rind extract to determine the pattern of the stains. Extracts from each sample were analyzed by TLC method using eluents with specific ratios, namely n-hexane:ethyl acetate (7:3), (9:1), and chloroform:methanol (9:1) eluents. The stationary phase was silica gel 60GF254, coated on an aluminum plate. The TLC chromatogram was sprayed using a 2% cerium sulfate solution. The stain contains

phenolics if the resulting stain is yellow after being sprayed with a 2% cerium sulfate solution.

Based on Fig. 4a, the n-hexane extract had four separation spots with an Rf1 value of 0.476 faded purple, Rf2 0.714 dark purple, Rf3 0.793 yellow, and Rf4 0.888 faded purple. The ethyl acetate extract had four separation spots with an Rf1 value of 0.079 yellow, Rf2 0.476 yellow, Rf3 0.555 dark purple, and Rf4 0.746 yellow. The methanol extract did not have a separation pattern or no elution (higher polarity).

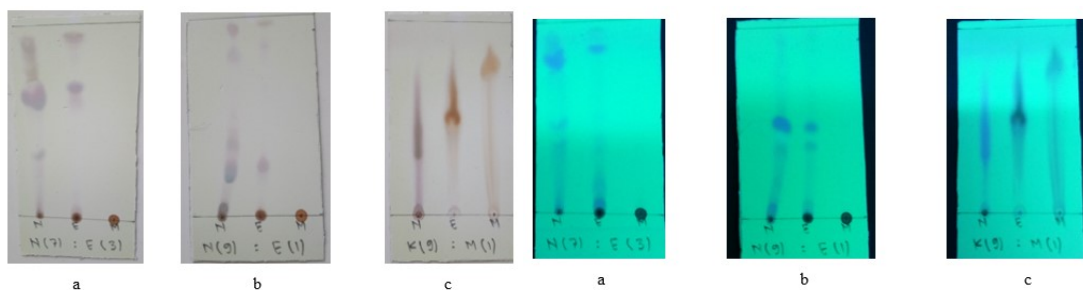


Figure 4. TLC chromatogram of white Kapul fruit rind extract with eluents of n-hexane:ethyl acetate (7:3) (a), (9:1) (b) and chloroform:methanol (9:1) (c) with 2% cerium sulfate stain (left) and 254 nm UV light (right)

Based on Fig. 4b, the n-hexane extract had five separation spots with Rf1 value of 0.079 black (terpene), Rf2 0.138 blue (steroid), Rf3 0.238 yellow (phenolic), Rf4 0.634 faded purple (terpene), and Rf5 0.714 colored yellow (phenolic). The ethyl acetate extract had three stains with an Rf1 value of 0.317, which was deep purple (terpene), Rf2 of 0.714 was pale purple (terpene), and Rf3 of 0.793 was yellow (phenolic). The

ethyl acetate extract was visible due to the presence of compounds that had not been eluted. The separation pattern was not found in the methanol extract, meaning that no compound was eluted (higher polarity). It proves that the compound content of each extract varies based on the polarity of the solvent. Based on Fig. 4c, the n-hexane extract only had one stain with an Rf1 value of 0.555, black (terpene). The ethyl acetate

extract with an Rf1 value of 0.428 was dark brown (phenolic), while the methanol extract had an Rf1 value of 0.650 faded brown (phenolic).

3.4. Compounds identification using the GC-MS instrument

Ethyl acetate extract from white Kapul fruit rind, which had the highest activity in the toxicity test, was analyzed using GC-MS SHIMADZU QP2010S with a 5 ms restex column, 30 m long, and ID 0.25 mm. The GC-MS analysis of the components of ethyl acetate extract of white Kapul fruit rind is presented in Fig. 5.

GC-MS analysis showed 32 peaks at retention times from 25,384 to 65,725. The four prominent compound peaks with the largest percentage area, namely 58.09%, were identified as ginoluton compounds with SI (Similarity index) 83, compounds 15-chloro-4-pentadesuna (16.25 %) with SI 81, compounds 17-(acetyloxy)- 2-methyl-, (2.α.,5. .,17. .)-estra-3-one (6.07%) with SI 80, and the compound methyl-11-octadesenoate (5.98 %) with SI 93. Compound components with SI values close to 100% indicate that the identified compounds are getting closer to the comparison compounds (Rasyid, 2016).

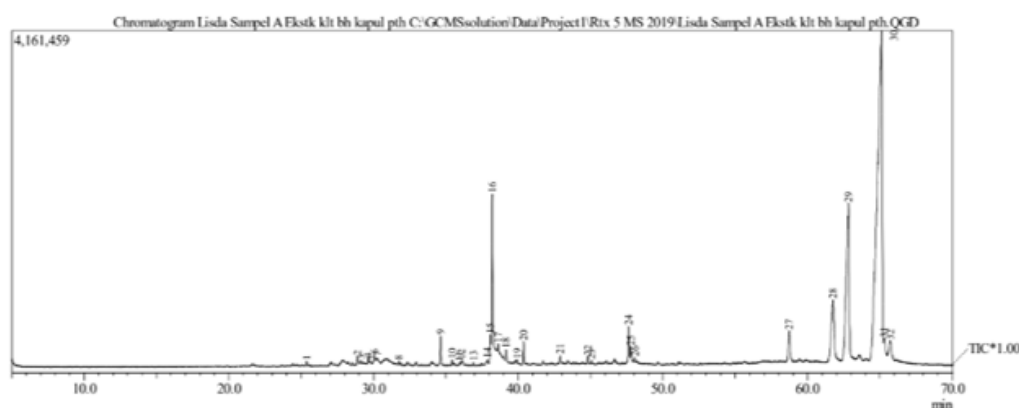


Figure 5. GC chromatogram of ethyl acetate extract of white Kapul fruit rind

Ginoluton is the main progestational steroid compound secreted mainly by the corpus luteum and placenta. Ginoluton acts on the uterus, mammary glands, and brain. Function in embryo implantation, pregnancy maintenance, and milk tissue development for milk production. Ginoluton is converted from pregnenolone. It also functions as an intermediate in the biosynthesis of gonadal steroid hormones and adrenal corticosteroids. Ginoluton is a progestin or synthetic form of the female sex hormone, namely progesterone. Ginoluton tricks the body into thinking that ovulation has occurred by maintaining high levels of synthetic progesterone. It prevents the release of the egg from the ovary. Ginoluton is used as a contraceptive. It shows that white Kapul fruit rind has the potential as a natural contraceptive drug.

The compound 15-chloro-4-pentadesuna shows potential activity in therapy against various diseases (Sivakrishnan, 2019). 15-chloro-4-pentadesuna also has anti-metastatic or anti-cancer effects (Kokila, 2014). The compound 17-(acetyloxy)-2-methyl-, (2.α.,5. .,17. .)-estra-3-on has an antioxidant effect (Erukainure, 2018).

Methyl dodecanoate, commonly called methyl laureate, is used as a raw material for methyl ester sulfonate (MES) detergents. MES is widely used in agricultural products to prevent the development of mosquito larvae. Methyl laurate or methyl esters are generally found in transesterification products in vegetable oils, such as the process of using alcohol (ethanol and methanol) with the help of a catalyst to break vegetable oil molecules into ethyl or methyl esters with glycerol as

a by-product (Arbianti, 2008). In the body, methyl laurate functions as an antiviral, antiprotozoal, and antimicrobial, improving the body's immune system, such as protection from HIV, herpes, and pathogenic bacteria (Adyana, 2017).

4. CONCLUSION

This study revealed that the ethyl acetate extract of white Kapul fruit rind was the most toxic to the BSLT test, with an LC₅₀ value of 350.87 ppm. The TLC chromatogram of each extract showed a different stain pattern according to the polarity of the solvent used. GC-MS analysis of the ethyl acetate extract of Kapul fruit rind showed the presence of 32 peaks, with the main compound being ginoluton (58.09%).

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