



Received on 13 January 2019; received in revised form, 11 April 2019; accepted, 19 April 2019; published 01 May 2019

ACTIVE COMPOUND FROM n-HEXANE FRACTION OF RAMPAI (*LYCOPERSICON ESCULENTUM*) LEAVES ETHANOL EXTRACT

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Keywords:

Active compound,
Triterpenoid, Cycloartenol, Hair
growth, *Lycopersicon esculentum*

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ABSTRACT: *Lycopersicon esculentum* leaves had traditionally been used to stimulate hair growth in Lampung Pesisir tribes, Tanggamus Regency, Lampung, Indonesia. This study aimed to examine the active compounds from the n-hexane *L. esculentum* leaves. The study based on our previous research showing that the n-hexane had hair growth stimulating effects. The study started with sample collection, extracted by maceration, fractionated using liquid-liquid extraction (LLE) with water, n-hexane and ethyl acetate with a ratio of 1: 1. Isolation of compounds in n-hexane fraction was done by vacuum liquid chromatography (VLC) method using gradient polarity of chloroform: methanol resulting in a subfraction forming a crystal. The crystal was checked by two ways TLC. The obtained pure crystal was then identified with FTIR, LC-MS, and NMR. The identification of the active compound isolates from the n-hexane isolates contained a class of triterpenoid compounds, namely cycloartenol compounds with the molecular formula $C_{30}H_{50}O$, m/z 425, 3783 with the name IUPAC 9 beta, 19-cyclo-24-lanostane-3beta-ol, (3beta) -9, 19-Cyclolanost-24-en-3-ol. The chemical compound content of n-hexane fraction of *L. esculentum* leaves with possibly causing anti-alopecia was the cycloartenol compound with the formula of $C_{30}H_{50}O$. Further verification determination is suggested to compare this compound to the minoxidil compound, either by *in-silico* or *in-vivo* analysis.

INTRODUCTION: *Lycopersicon esculentum* is included in the family Solanaceae, has a synonym for *S. lycopersicum*, *Lycopersicum esculentum*, *Lycopersicon pimpinellifolium*, *Solanum esculentum* and *Lycopersicon lycopersicum*^{1, 2, 3, 4}. The species originated in Western South America. In Indonesia, these plants are spread throughout the Indonesian archipelago.

Young fruit has a bitter and flavorful taste because it still contains lycopersin in the form of mucus. The unpleasant aroma will disappear by itself when the fruit enters the ripening phase until it ripens. It will also turn into a slightly sour sweetness that characterizes the taste of the potpourri.

In the general public, the potpourri is only made as a vegetable without any more use, like flour, for example, an alternative food source can be made considering that the nutrition it contains is quite complex, even though the fruit after harvest will be damaged between 20% and 50% after harvest⁵. Lycopene from tomatoes⁶ including *L. esculentum* fruit can inhibit the growth of endometrial cancer, breast cancer, and lung cancer in cell cultures with

	<p>QUICK RESPONSE CODE</p>
	<p>DOI: 10.13040/IJPSR.0975-8232.10(5).2537-44</p>
<p>The article can be accessed online on www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(5).2537-44</p>	

higher activity compared to α and β -carotene⁷. Lycopene as a carotenoid provides radioprotectant and antioxidant effects by quenching radiation-induced free radical singlet oxygen⁸. Tomato leaves contain toxic glycoalkaloids which if consumed by humans in large quantities can cause difficulty breathing, nausea, vomiting, so people are reluctant to use it⁹. In addition to the mineral content, tomatoes also contain peroxidase enzymes obtained from fruit, leaves and stem tissue from tomatoes¹⁰. Tomato leaves contain peroxidase, and together with H₂O₂, these compounds can be converted into hypothiocyanite (OSCN⁻) which has antimicrobial activity¹¹. This compound can be used as an alternative as a food preservative by using it as an antimicrobial agent.

Lampung Pesisir tribe, Lampung Province, West Indonesia traditionally uses *Lycopersicon esculentum* Mill leaves. They call it as Rampai leaves as fertilizer for crushed hair, applied to the scalp twice a day¹². Previously, we reported a research article called *in-vivo* hair growth stimulating the activity of ethanol extract and its fractions from Rampai Lampung (*Lycopersicon esculentum* Mill.) leaves and found that n-hexane fraction had growth hair activity better than other fractions compared to minoxidil as a positive control¹³. This study reports scientific data and information about the compounds contained in the *L. esculentum*-hexane fraction to search for an anti-alopecia compound. So far in the pharmacological handling of baldness, the Food and Drug Administration (FDA) only allows two drugs, minoxidil, and finasteride^{14,15}.

MATERIALS AND METHODS:

General: Moisture balance (Metler Toledo), Rotary evaporator (Buchi R II), spectroscopy FT-IR (Shimadzu 8400), Spectrophotometer UV-Vis (Shimadzu UV 1800 U), Mass-spectroscopy LC-MS Shimadzu QP-2010. Nuclear magnetic resonance (NMR) Agilent 500 MHz with a DD2 console system, operating at 500 MHz (1H) and 125 MHz (13C), DEPT and NMR-2D technics including HMQC dan HMBC. Column chromatography (CC) was carried out using silica gel (E. Merck), 70-230 mesh) and Sephadex LH-20 (Sigma-Aldrich). All the reagents and solvents used for separation and purification were analytical grade and locally purchased.

Plant Materials: The plant material used in the form of rampai (*L. esculentum*) leaves were obtained from cultivation plantations in Gisting District, Tanggamus Regency, Lampung Province and determined at the Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia.

Extraction of the *L. esculentum* Leaves: The extraction of the rampai leaves was maceration method refers to the existing standard method^{16,17}. The rampai leaves were incorporated into a cotton-coated macerator, 70% ethanol added to all crushed and dried sample for 3 × 24 h while stirring occasionally. The extract was then evaporated and concentrated by a rotatory evaporator at 60 °C until the solvent evaporated almost entirely, then the extract was stored on the vapor plate and thickened over the water bath until a concentrated extract was obtained.

Fractionation of Ethanol Extract: Fractionation was done by using Liquid-Liquid Extraction (LLE). The fractionation was performed by using n-hexane and ethyl acetate solvents. The n-hexane fraction was thickened by using a rotary evaporator followed by heating on a water bath^{18,19,20}.

Phytochemical Screening: Phytochemical Screening to know secondary metabolite content of leaves ethanol extract and n-hexane fraction was based on the Farnsworth method²¹.

Vacuum Liquid Chromatography (VLC): VLC was used to separate and isolate desired fractions. The fractional water fraction was introduced into the VLC column and eluted with methanol: chloroform mixture with multilevel polarization. Each sub-fraction was tested by TLC with a suitable developer to obtain a spot. The TLC used a stationary phase of silica gel GF254 and its mobile phase of chloroform: methanol with a variation. The VLC method was guided by Indonesian Herbal Pharmacopoeia²².

Purification and Purity Isolate Test: Purification was done by re-crystallization using methanol. The crystals contained in the vials were dissolved with methanol, and the exterior of the vial was soaked in warm water with a temperature range below 40 °C while rocking slowly. Then, directly inserted into

cold water, not to be submerged or water went into the vial. The isolate purity was tested by using two-way TLC. The stationary phase was used in the form of silica gel 60 with two different mobile phase systems.

Isolate Identification: The resulting isolates were then identified and determined by molecular structure by physics, TLC with spray reagent, ultraviolet-visible spectrometry (UV-Vis), IR spectrometry, LC-MS, Proton (H-NMR) and carbon (C13-NMR), DEPT with 2D-NMR techniques including HSQC, HMBC, and COSY. These methods referred to standards Elucidation Structure of Organic Compound textbooks^{23,24}.

RESULTS AND DISCUSSION:

Extraction and Fractionation: It was obtained that the yield of ethanol extract and n-hexane fraction was 12.91% w/w (from dried leaves) and 10.5% w/w (from ethanol extract), respectively.

Phytochemical Screening: To determine the secondary metabolites contained in extracts of rampai leaves and n-hexane fractions, phytochemical screening was carried out. Phytochemical screening results of n-hexane fraction and leaf ethanol extract can be seen in **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING RESULTS OF ETHANOL EXTRACTS AND n-HEXANE FRACTIONS OF RAMPAI LEAVES

Secondary metabolites	Results	
	Ethanol extract ¹²	n-hexane fraction
Alkaloids	+	+
Flavonoids	+	+
Saponin	+	+
Polyphenols	-	-
Tannins	-	-
Quinone	+	-
Monoterpenoids and Sesquiterpenoids	+	+
Steroids dan Triterpenoids	+	+

Notes: (+): detected; (-): not detected

Our previous report¹³ discussed and stated that the difference in the results of the phytochemical screening, was probably due to differences in plant origin.

Vacuum Liquid Chromatography (VLC)

Results: VLC was chosen as one of the fraction initial separation methods due to the effectiveness

of fraction separation and faster time compared to ordinary chromatography. With the help of a vacuum, the pressure in the chromatographic system was lower than the pressure of the surrounding environment so that the eluent was more quickly pushed out of the column and the elution process runs faster. The principle of VLC was almost the same as other chromatography, *i.e.* compounds with a higher level of polarity would be bonded or held longer for the stationary phase of the silica gel G60. Compounds with lower levels of polarity would elute first so that the separation of these compounds could occur based on polarity. To cover the polarity, two types of eluents were applied (see **Table 2**), so 21 subfractions were obtained.

TABLE 2: COMPARISON OF ELUENTS IN VLC

S. no.	Eluents			
	A		B	
	n-hexane	Ethyl acetate	Ethyl acetate	Methanol
1	200	0	180	20
2	180	20	160	40
3	160	40	140	60
4	140	60	120	80
5	120	80	100	100
6	100	100	80	120
7	80	120	60	140
8	60	140	40	160
9	40	160	20	180
10	20	180	0	200
11	0	200	-	-

Each subtraction was examined TLC by eluent n-hexane and ethyl acetate (9: 1). The same spots were then collected using spotting appearance in the form of Detection (a) visible light, (b) UV light λ 254 nm, and (c) UV λ 366 nm, 10% H₂SO₄ spray reagent.

Re-crystallization: The results of the re-crystallization were obtained by white deposits which were then re-checked by TLC. The crystal was analyzed by physical and instruments.

Physics Examination Results with Melting

Distance Test: Testing the purity of the isolate in the form of white solids was carried out to determine the melting distance. The initial temperature for melting distance testing is 100 °C from the results of the test results obtained that the melting distance of solid white isolates was 100.2 °C - 100.3 °C. The melting distance inspection

results indicated that the isolates were entirely pure because the temperature difference range was quite narrow, namely 0-1°C. MP suspected compounds was 99 °C²⁵. Melting distance plays an essential role in the process of identifying and testing the purity of a solid organic compound. Pure compounds had a sharp melting distance between the temperature spacing of these compounds is very small when it changed perfectly from solid to liquid. The maximum temperature distance for pure compounds is 1-2 °C. This method was important for purity testing because the fact shows that impure compounds had a low melting temperature and wider melting distance. Therefore, the first purity test that must be done was the determination of melting distance²⁶.

UV-Vis spectrometry was used to measure obtained white amorph crystal based on wavelength to determine electron absorbance and transition²³. The identification was carried out by UV-Vis spectrophotometry at a wavelength of 200-500 nm, with a concentration of 100 ppm which was as much as 1 mg in 10 ml of methanol. The results of the UV spectrum of the isolated compounds showed that there were two maximum absorbances which appeared, namely at 223 and 272 nm. The wavelength that appeared on 223 and 273 uptakes was the wavelength originating from the chromophore ethylene band caused by an electronic transition $\pi \rightarrow \pi^*$ in the side chain. It was known that the maximum wavelength at 278 nm with an absorbance of 0.2888, it was known that the extension coefficient (ϵ) was 1,224.51 so that compounds with the molecular formula C₃₀H₅₀O had a double bond. To find out the number of double bonds possessed by compounds of the crystal could be calculated using the formula:

$$DBE = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{N}{2}$$

Where: C= Number of Carbon atoms present; H = Number of Hydrogen atoms present; X= Number of Halogen atoms present (Cl, Br, I or F); N= Number of Nitrogen atoms present; DBE = Double bond equivalent.

From the calculations with the above formula, it was known that the number of double bonds of C₃₀H₅₀O compounds was as many as 6 pieces.

Infra-Red (IR) Spectrometry: Infrared spectroscopy was chosen because it could determine the functional groups of a compound by determining the atomic vibrations of a molecule at each wavenumber²³. The results of the IR spectrum of the obtained crystal can be seen in **Table 3** and **Fig. 1**.

TABLE 3: IR SPECTRA OF THE OBTAINED CRYSTAL

Wave numbers (cm ⁻¹)	Intensity	Peak shapes	Interpretation
3346.8	Moderate	Wide	- OH
2929.2	Strong	Sharp	C-H sp ²
2868.8	Strong	Sharp	C-H sp ³
1662.8	Strong	Sharp	C=C
1456.7-1376.6	Strong	Sharp	gem-dimethyl
1046.6-1024.7	Strong	Sharp	C-O

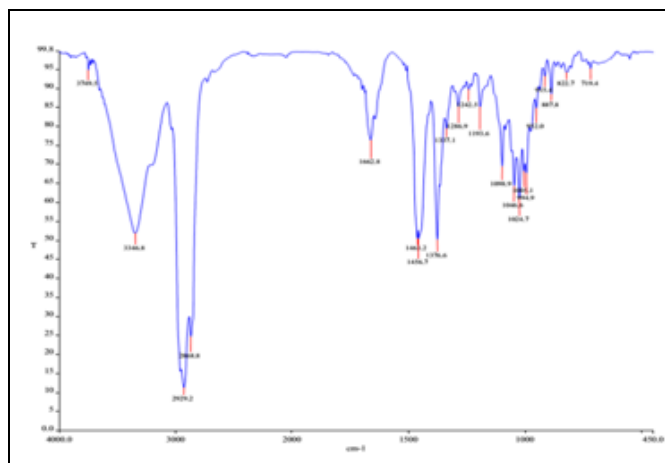


FIG. 1: IR-SPECTRA OF THE OBTAINED CRYSTAL

LC-MS Examination: Based on the LC-MS HR-TOFMS spectrum **Fig. 2** it was known that m/z 426.3751 [M + H]⁻, and the mass calculation of the crystal was m/z 425, 3783.

¹H Spectrometry and ¹³C-NMR:

A. ¹H and ¹³C-NMR: NMR spectroscopy was used to determine the structure of organic compounds based on information from the spectrum produced so that it could combine functional groups into structural units²⁶. It was found that the ¹H-NMR spectrum of the isolate compound showed the presence of fifty protons, including seven methyl signals that resonate at δ H 0.83 (3H, s, H-31), 0.87 (3H, s, H-30), 0.90 (3H, s, H-18), 0.92 (3H, s, H-32), 0.96 (3H, d, 7.0 H-21), 1.59 (3H, s, H-26) and 1.70 (3H, s, H-27) which indicated the characteristics of the cycloartane triterpenoids compound, one metin oxygenated at δ H 3.28 (1H, m, H-3), one metin sp² at δ H 5,10 (1H, m, H-24).

The ^{13}C -NMR spectrum of the isolate compound **Fig. 3** detailed by the 135° DEPT experiment shows the presence of 30 carbon signals consisting of seven methyl, eleven methylene, six metin, and six quaternary carbon signals that indicate group triterpenoid compounds sikloartan. The presence of seven methyl signals that resonated at δc 14.2 (C-31), 17.8 (C-26), 18.2 (C-21), 18.4 (C-18), 19.5 (C-32), 25.6 (C-30), and 25.9 (C-27), one oximetin sp^2 signal at δc 79.0 (C-3), one sp^2 quaternary carbon

signal at δc 131.0 (C- 25), one metin sp^2 signal at 125c 125.4 (C-24), five sp^3 quaternary carbon signals at δc 20.2 (C-9), 26.2 (C-10), 40.6 (C-4), 45.4 (C-13) and 48.9 (C-14), eleven methylene sp^3 signals at δc 21.3 (C-6), 25.1 (C-23), 26.2 (C- 11), 26.6 (C-7), 28.3 (C-16), 30.1 (C-19), 30.6 (C-2), 32.1 (C-12), 33, 1 (C-1), 35.7 (C-15) and 36.5 (C-22), four signals metin sp^3 at δc 36.0 (C-20), 47.2 (C-8), 48, 1 (C-5) and 52.4 (C-17).

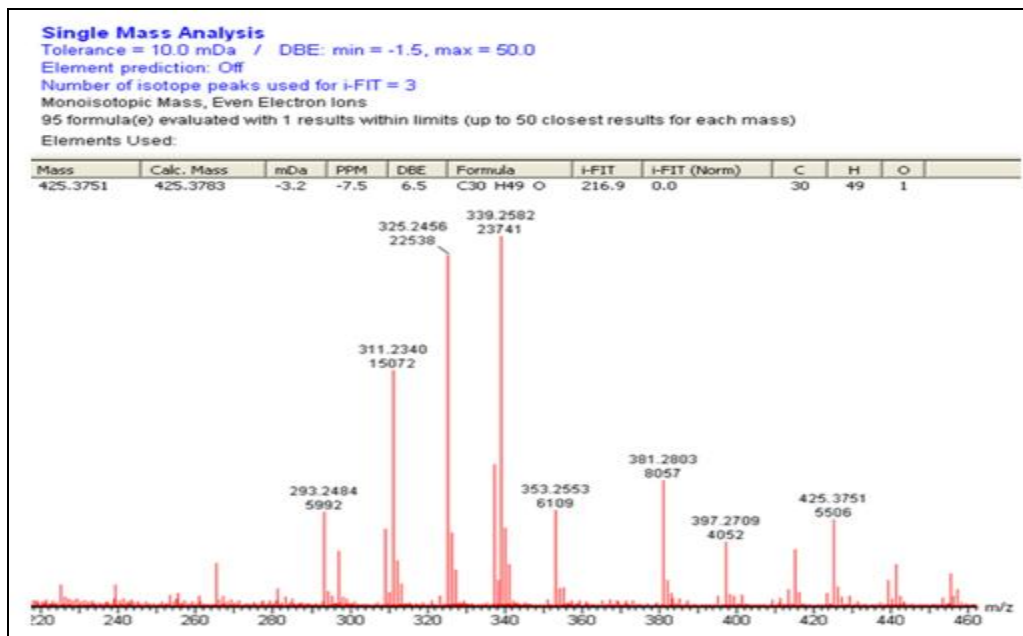


FIG. 2: HR-TOFMS SPECTRA OF THE OBTAINED CRYSTAL

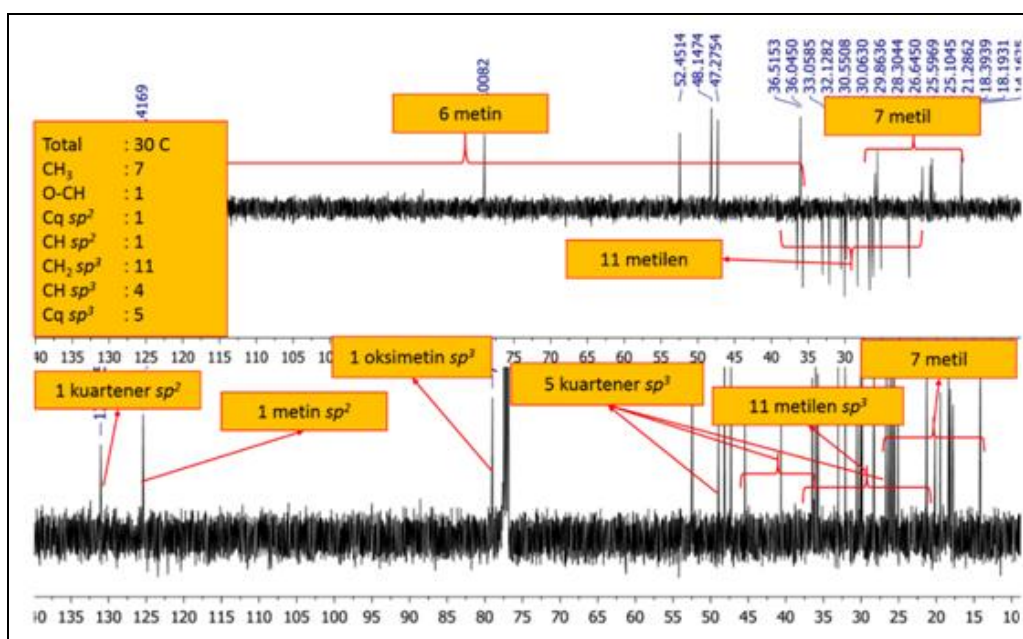


FIG. 3: ^{13}C -NMR SPECTRA AND DEPT 135° OF OBTAINED CRYSTAL (125 MHz IN CDCl_3)

B. DEPT: Based on the results of the ^{13}C -NMR spectrum measurements in **Fig. 3**, it could be

observed that there were seven methyl carbons, eleven methylene carbon, six methine carbon and

six carbon quaternary. So that the isolate consisted of carbon atoms that number thirty carbon, fifty hydrogens where one hydrogen comes from hydroxyl and 1 oxygen which was a typical framework of triterpenoid compounds in the cycloartane group²⁸.

C. HSQC, HMBC, and COSY: Ownership of protons to carbon was determined based on HSQC experiments. Methyl and methine were characterized by red patches while methylene was marked with blue patches. There was a correlation of three protons to one methyl carbon fruit that is between methyl protons H3-18 to C-18, H3-21 to C-21, H3-26 to C-26, H3-27 to C-27, H3-30 to C-30, H3-31 to C-31 and H3-32 to C-32 shows H3-18 bound to C-18, H3-21 bound to C-21, H3-26 bound to C-26, H3-27 bound to C-27, H3-30 bound to C-30, H3-31 bound to C-31 and H3-32 bound to C-32.

Two protons against one methylene carbon fruit are between methylene protons H2-1 to C-1, H2-2 to C-2, H2-6 to C-6, H2-7 to C-7, H2-9 to C-9, H2-11 to C-11, H2-12 to C-12, H2-15 to C-15, H2-16 to C-16, H2-22 to C-22 and H2-23 to C-23 showed two protons namely H2-1 bound to C-1, H2-2 bound to C-2, H2-5 bound to C-5, H2-6 bound to C-6, H2-9 bound to C-9, H2-11 bound to C-11, H2-12 bound to C-12, H2-15 bound to C-15, H2-16 bound to C-16, H2-22 bound to C-22 and H2-23 bound to C-23. One proton to one carbon meth H-3 to C-3, H-5 to C-5, H-8 to C-8, H-17 to C-17, H-20 to C-20, and H-24 to C-24 shows one proton, H-3 bound to C-3, H-5 bound to C-5, H-8 bound to C-8, H-17 bound to C-17, H-20 bound at C-20, and H-24 bound to C-24. The correlation of each methyl group showing the triterpenoid framework of the cycloartane group was determined based on the HMBC experiment. There is a correlation between methyl H3-30 and H3-31 protons with C-3, C-4, C-5, and C-30 / C-31, indicating that ring A has dimethyl and oxymethine gems. Correlation between proton methyl H3-26 and H3-27 with C-25, C-24 which was olivenin and C-26 / C-27 shows side chains that had dimethyl gems bound to carbon that had bonds π .

Correlation between methyl H3-18 and H3-32 protons with C-12, C-13, C-14, C-17, C-8, C-13, C-14, and C-15 showed the methyl group H3-18, and H3-32 was located parallel to that of carbon C-13

and C-14. Correlation of methyl H3-21 protons with C-20, C-17, and C-22 showed that the methyl group H3-21 was in the side chain while proving the side chain substitute was bound at C-17 in the cycloartane triterpene framework. The correlation of the whole methyl proves that the A, B, C, and D rings were intact. Determination of the location of the hydroxyl group on ring A was confirmed by the HMBC experiment. There was a correlation between methyl H3-30 and H3-31 protons, with C-3, methylene H2-1 and H2-2 with C-3 and the correlation of methine H-5 with C-3 showed that the hydroxyl group was in the position of rings A and C-3. From 1H-1H COSY the correlation between H-1 / H-2 / H-3, H-5 / H-6 / H-7 / H-8, H-11 / H-12, H-15 / H-16 / H-17 / H-18, H-19 / H-20 / H-21 / H-22 / H-23 and showed that crystal compounds were cycloartane triterpenoid compounds.

The following data on chemical shift 1H-NMR and ¹³C-NMR isolate compounds are shown in **Table 4**. To strengthen the suspicion of the structure of the isolated compound, further literature studies were carried out by comparing data with analogous compounds with similar frameworks. The chemical shift data of this obtained crystal was compared with the Cycloartenol compound²⁹.

Fig. 4 showed the structure of cycloartenol which was thought to be the crystal compound obtained. Determination of the presence of cycloartenol in the ethanol extract of *L. esculentum* leaves has not been published elsewhere., but the presence of cycloartenol in potatoes²⁹ and jackfruit³⁰ has been reported by others using different techniques.

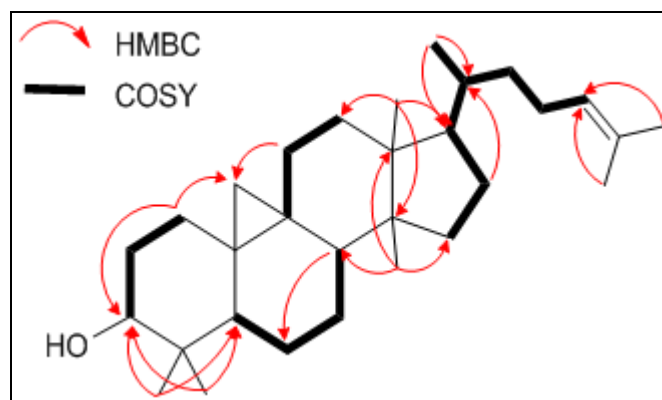


FIG. 4: STRUCTURE AND CORRELATION OF HMBC AND 1H-1H COSY OF OBTAINED CRYSTAL/ CYCLOARTENOL

TABLE 4: DATA ON CHEMICAL SHIFTS OF ¹H-NMR AND ¹³C-NMR OBTAINED CRYSTAL COMPARED TO STANDARD CYCLOARTENOL COMPOUNDS

	Obtained crystal		Cycloartenol	
	¹³ C-NMR	¹ H-NMR	¹³ C-NMR	¹ H-NMR
	δ_c /ppm	δ_c (Mult, J/Hz)	δ_c /ppm	δ_c (Mult, J/Hz)
1	33,1	1,63 (m) 1,55 (m)	32,0	1,25 (m) 1,55 (m)
2	30,6	1,58(m) 1,77 (m)	30,4	1,57 (m) 1,75 (m)
3	79,0	3,28 (m)	78,9	3,28 (m)
4	40,6	-	40,5	-
5	48,1	1,52 (dd, 12,5; 4,9)	47,2	1,29 (dd, 12,5; 4,9)
6	21,3	1,57 (m) 1,61 (m)	21,1	0,79 (m) 1,59 (m)
7	26,6	1,13 (m) 2,02 (m)	26,0	1,08 (m) 1,32 (m)
8	47,2	1,30 (dd, 12,5; 4,9)	48,0	1,51 (dd, 12,5; 4,9)
9	20,2	-	20,1	-
10	26,2	-	26,1	-
11	26,2	1,06 (m) 1,34 (m)	26,5	1,11 (m) 1,99 (m)
12	32,1	1,25 (m) 1,28 (m)	33,0	1,62 (m)
13	45,4	-	45,3	-
14	48,9	-	48,8	-
15	35,7	1,29 (m)	35,6	1,28 (m)
16	28,3	1,29 (m) 1,90 (m)	28,2	1,28 (m) 1,90 (m)
17	52,4	1,60 (m)	52,3	1,59 (m)
18	18,4	0,90 (s)	18,0	0,97 (s)
19	30,1	0,36 (d, 4,2) 0,58 (d, 4,2)	29,9	0,33 (d, 4,2) 0,56 (d, 4,2)
20	36,0	1,39 (d, 7,0)	35,9	1,38 (m)
21	18,2	0,96 (d, 7,0)	18,3	0,89 (d, 7,0)
22	36,5	1,05 (m) 1,45 (m)	36,4	1,05 (m) 1,44 (m)
23	25,1	1,87 (m) 2,06 (m)	25,0	1,86 (m) 2,04 (m)
24	125,4	5,10 (m)	125,3	5,10 (m)
25	131,0	-	130,9	-
26	17,8	1,59 (d)	17,6	1,68 (d)
27	25,9	1,70 (s)	25,7	1,61 (s)
30	25,6	0,87 (s)	25,5	0,97 (s)
31	14,2	0,83 (s)	14,0	0,81 (s)
32	19,5	0,92 (s)	19,3	0,89 (s)

¹³C-NMR (125 MHz dalam CDCl₃)¹H-NMR (500 MHz dalam CDCl₃)

CONCLUSION: The leaves of *L. esculentum* were popularly used by Lampung Pesisir tribes, Lampung Province, West Indonesia for hair growth treatment, and such traditional application awakened our research interest. Our previous pharmacological evaluation and phytochemical screening of the leaves helped clarify its active ingredients.

Our investigation has led to the isolation of triterpenoid compounds namely cycloartenol. This result enriches the chemical constituents of *L. esculentum* leaves.

ACKNOWLEDGEMENT: This work was financially supported by the ALG (Academic Leadership Grant) Universitas Padjadjaran.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

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How to cite this article:

Oktoba Z, Moektiwardoyo M and Mustarichie R: Active compound from n-hexane fraction of Rampai (*Lycopersicon esculentum*) leaves ethanol extract. Int J Pharm Sci & Res 2019; 10(5): 2537-44. doi: 10.13040/IJPSR.0975-8232.10(5).2537-44.

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