



## Synthesis, Characterization, Anticancer And *In silico* ADME Properties Of Caproic Acid Derivatives Against P388 Cancer Cell Lines

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### Abstract

The *in silico* properties of absorption, distribution, metabolism, and excretion (ADME) and drugs likeness of caproic acid ester derivatives were evaluated. The esterification of caproic acid from palm kernel oil with citronellol and geraniol from citronella oil has been carried out using base (NaOH) catalyst. The cytotoxicity of ester compounds were also tested against P388 murine leukemia cancer cell lines using MTT method. Analysis of the ester products were carried out using spectroscopic (IR, MS and <sup>1</sup>H-NMR) methods, which were confirmed that the desired compounds were successfully synthesized. Caproic acid ester derivatives showed higher anticancer activity compared to the parent caproic acid for P388 cancer cell lines. Citronellyl caproate with  $IC_{50}$   $4.15 \times 10^{-2}$   $\mu$ M has the stronger activity than geranyl caproate with  $IC_{50}$   $11 \times 10^{-2}$   $\mu$ M. ADME studies for the synthesized compounds revealed that the ester compounds have fulfill the Lipinski rule of five and has no carcinogenicity effect to rat or mouse.

**Keywords:** ADME, caproic acid, ester, P388, MTT

## Sintesis, Karakterisasi, Sifat Antikanker dan ADME *In silico* dari Turunan Asam Kaproat pada Sel Kanker P388

### Abstrak

Studi *in silico* terhadap absorpsi, distribusi, metabolisme, dan ekskresi (ADME) serta sifat kemiripan obat (*drug likeness*) ester turunan asam kaproat telah dilakukan. Esterifikasi asam kaproat dari minyak inti sawit dengan sitronelol dan geraniol dari minyak sereh dilakukan dengan menggunakan katalis basa (NaOH). Uji sitotoksitas senyawa ester dilakukan terhadap sel kanker murine leukemia P388 menggunakan metode MTT. Analisis produk ester yang dilakukan menggunakan metode spektroskopi (IR, MS dan <sup>1</sup>H-NMR), menunjukkan bahwa senyawa ester yang diinginkan berhasil disintesis. Ester turunan asam kaproat menunjukkan aktivitas antikanker yang lebih tinggi dibandingkan dengan senyawa induk asam kaproat. Sitronelil kaproat dengan  $IC_{50}$   $4,15 \times 10^{-2}$   $\mu$ M memiliki aktivitas antikanker lebih kuat daripada geraniol kaproat dengan  $IC_{50}$   $11 \times 10^{-2}$   $\mu$ M. Studi ADME secara *in-silico* terhadap senyawa ester yang disintesis menunjukkan bahwa senyawa ester tersebut telah memenuhi aturan lima Lipinski dan tidak memiliki efek karsinogenik terhadap tikus.

**Kata Kunci:** ADME, asam kaproat, ester, P388, MTT

## 1. Introduction

Cancer is a deadly disease where cells divide in an uncontrolled manner and it can develop in any part of the body in any form such as liquid and solid.<sup>1</sup> In 2012, within 5 years of diagnosis, there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer in worldwide. In South East Asia there were 1724 thousands cases and 1171 thousands cancer death. Cancer disease was one of cause of human death in the world, and in Indonesia, cancer is a second largest killer after heart disease.<sup>2,3</sup> Conventional therapies ranging from surgery, radiotherapy and chemotherapy still remains dangerous because of the limited efficacy of all the available approaches. Following complete treatment, cancer cells retain the property of metastasis and frequently recurred the patient often dies in secondary effect rather than the cancer disease itself. Thus, there is an urgent for developing efficient drugs specific for cancer with no affect to normal cells but effective against cancer cells only.

During the last few decades, chemopreventive and chemotherapeutic compounds against various types of cancer have been isolated from a number of medicinal plants.<sup>4,5,6</sup> Indonesian biodiversity has potential as a source of bioactive compounds as chemopreventive and chemotherapeutic agent for cancer treatment. Beside it, in Indonesia, almost all the raw materials for synthetic medicine are still imported until now. Therefore, it is necessary to utilize the potential of local natural compounds as raw materials for synthesis of medicine as chemopreventive and chemotherapeutic agent for cancer treatment. In this study, we synthesis of ester compounds from Indonesian native plants, caproic acid that derived from palm kernel oil (PKO) with citronellol and geraniol from citronella oil. Caproic acid (C<sub>6</sub>) that can be isolated from PKO was reported have bioactivity as antimicrobial, antifungal, antiviral and potentially as anti-neoplastic agents.<sup>7,8</sup> Indonesia is the second palm oil producer in the world, after Malaysia.<sup>9</sup> Citronella oil is derived from Lemongrass

plant that's one of typical plants Indonesia including in genus of *Cymbapogon* and family of Poaceae.<sup>10</sup> Citronella oil is one of the main commodities of various essential oils in world trade, and Indonesia is the third citronella oil producer in the world, after China and Vietnam.<sup>11</sup>

Here in this paper we report 2 caproic acid ester derivatives and evaluated their anticancer properties against murine leukemia (P388) cancer cell lines. The pharmacokinetic parameters (absorption, distribution, metabolism and excretion/ADME) and drugs likeness properties have also evaluated using preADMET web based software.<sup>12,13</sup>

In this study, the synthesis of citronellyl caproate and geranyl caproate were carried out by using sodium hydroxide (NaOH) as catalyst. Identification of ester compound was carried out by Thin Layer Chromatography (TLC), Fourier Transform Infra Red (FTIR), Gas Chromatography Mass Spectroscopy (GCMS) and Proton Nuclear Magnetic Resonance (<sup>1</sup>H-NMR) spectrometer. The *in vitro* cytotoxic activity of citronellyl caproate and geranyl caproate against murine leukemia (P388) cancer cells was carried out by using MTT method.

## 2. Methods

### 2.1. Materials

Materials used were citronellol, geraniol from Indonesian flavour and fragrance industry, PT Aroma Essence Prima with purity 90%, and caproic acid from Indonesian oleochemical industry, PT Sumi Asih with purity 99% as starting materials, NaOH (E.Merck 106469), 1% of HCl solution. All of solvents such as n-hexane, ethyl acetate (EtOAc) and methanol were distilled according to standard procedure. Analytical grade chemicals were used for mass and molecular structure spectroscopy, and also for *in vitro* cytotoxic activity analysis.

### 2.2. Instrument

Equipments used in this study were one unit of esterification process, one unit of evaporation process, and one set of ester compound identification unit. TLC was

performed on precoated silica gel plates (Kiesel gel 60 F254 0.25 mm), spots were visualized under UV light (254 and 366 nm) irradiation and by spraying using 10% of H<sub>2</sub>SO<sub>4</sub> solution followed by heating at 110°C. Silica gel column chromatography was carried out on Merck 64271 (70-230 mesh). IR spectra was measured on a FTIR Shimadzu prestige 21 using KBr pellets. Mass spectrum (MS) was measured by GC Agilent Technologies 7890B with 5977A MSD. <sup>1</sup>H-NMR spectrum was recorded on a NMR Jeol spectrophotometer for 500 Mhz in deuterio chloroform. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm) and downfield from CDCl<sub>3</sub> ( $\delta$  7.26).

### 2.3. Procedure

#### 2.3.1. Synthesis of citronellyl caproate and geranyl caproate ester compounds

A total of 1.56 g of citronellol or 1.54 g of geraniol was reacted with 1.39 g of caproic acid, and 69.7 mg of NaOH was added, and stirred for 8 hours at 80°C. NaOH as base catalyst was separated from synthesis product by using 1% of HCl solution. The synthesis product was extracted with EtOAc, washed with water (distilled water), and separated between water and EtOAc. The filtrate was concentrated by evaporating of EtOAc at 45°C under vacuum to obtain product containing of a mixture esterification product. The crude ester was analyzed by TLC with n-hexane : EtOAc = 95 : 5 as eluent.

#### 2.3.2. Purification of ester products

Ester product was purified by column chromatography (silica gel Merck 64271) eluting with n-hexane, a gradient of EtOAc to 100%. The pure ester product was identified by spectroscopic methods (IR, MS and <sup>1</sup>H-NMR).

#### 2.3.3. In-vitro cytotoxic activity analysis

The inhibitory effect of ester product on murine leukemia (P388) cells assessed using MTT (Mosmann) method with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colouring. The development of cells culture performed by

growing of cells culture in RPMI 1640- fetal bovine serum (FBS) medium and incubated in a humidified atmosphere of 37°C and 5% of CO<sub>2</sub> for 24 hours. Serum medium was replaced by new serum medium and incubated in a humidified atmosphere of 37°C and 5% of CO<sub>2</sub> again so further to obtain sufficient cells number for testing. After a sufficient number of cells, cell medium removed, and washed with phosphoric acid buffer solution (PBS), and then RPMI medium added. The cells were transferred into tubes and centrifuge at 1200 rpm for 5 minutes. Supernatant was discarded and the precipitate was added RPMI 1640 medium containing 10% of PBS. The cells density was calculated by hemocytometer and cells number was counted. The number of cells that known then made dilution by adding with RPMI-serum medium to obtain cell number of 2 X 10<sup>4</sup> cells/ml of cell suspension. Cells with densities 1-2 X 10<sup>4</sup> cells/well in 96 well-plates cultivated in a humidified atmosphere of 37°C and 5% of CO<sub>2</sub> for 24 hours. Afterwards the cell cultures were replaced, washed with PBS, and added with 100  $\mu$ l fresh culture medium containing sample at concentration of 100, 50, 25, 10, 5, 2.5, and 1  $\mu$ g/ml. Furthermore, the plates were incubated at 37°C in CO<sub>2</sub> 5% for 48 hours. At the end of the treatment, medium was then replaced and the cells were washed with PBS and added with 100  $\mu$ l of a new fresh medium containing MTT 5 mg/ml. Plates were incubated in a humidified atmosphere at 37°C in 5% CO<sub>2</sub> for 4 hours to bioreduction of the MTT dye into purple formazan crystals. After 4 hours, the medium containing MTT was discarded, washed with PBS, added 200  $\mu$ l isopropanoic solution, and then incubated at room temperature for 12 hours to completely solubilize the formazan crystals. The bioreduction of MTT was assessed by measuring the absorbance of each well at 550 nm by ELISA reader. Viable cells were expressed as a percentage of the absorbance treatment cells divided by absorbance control cells multiplied viable cell versus concentration. The IC<sub>50</sub> express 50% death induced by the concentration and was a measurement of the effectiveness of a compound in inhibiting biological function

ester.<sup>14,15</sup>

#### 2.3.4. Drug likeness and ADME properties prediction

Drug-likeness and ADME properties were calculated using web based software. In this study, the PreADMET program was used to predict ADME of caproic acid and its derivatives. The aspect prediction of absorption properties included percentage human intestinal absorption (% HIA) and Caco-2 cell permeability. Lipinski rules of five as a rule of thumb for drug likeness properties including log P (less than 5), hydrogen bond acceptors (less than 10), molecular weight (less than 500), and hydrogen bond donors (less than 5) has also calculated.<sup>12,13</sup>

### 3. Results

The crude ester product that obtained was oily liquid. The TLC analysis of synthesis product showed that synthesis product contain ester compound which was indicated by a spot with R<sub>f</sub> value of 0.42 of citronellyl caproate and 0.4 of geranyl caproate, lower than R<sub>f</sub> of citronellol (0.45), geraniol (0.8) and caproic acid (0.82) as starting material.

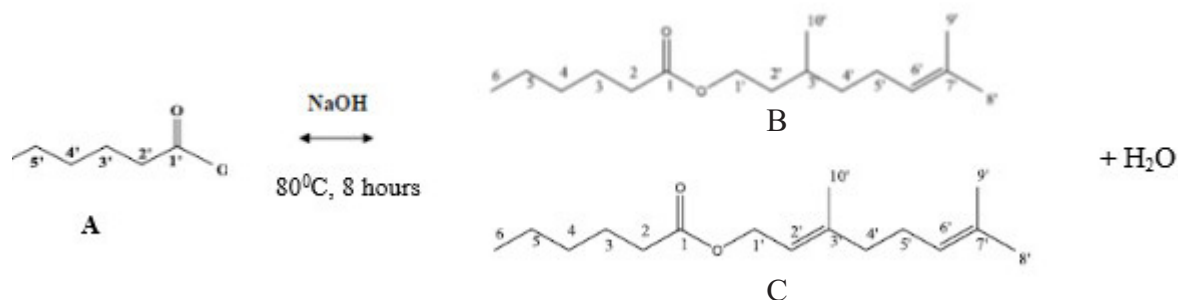
The crude ester further purified by using chromatography column using silica gel as the stationary phase and a gradient eluent system of n-hexane : EtOAc from 100% of n-hexane by 1% increment of EtOAc as the mobile phase. Pure citronellyl caproate and geranyl caproate esters were obtained by using eluent system solvent of n-hexane : EtOAc = 98 : 2. The weight and yield of pure citronellyl caproate and geranyl caproate were 1.29 g (50.48%) and 1.94 g (76.72%). The esterification of caproic acid with citronellol and geraniol as shown in Figure 1.

The IR spectra of citronellyl caproate compound in Figure 2A displayed the formation of a new group at wave number,  $\nu_{\max} = 1728.22 \text{ cm}^{-1}$  which is stretching vibration carbonyl (C=O) of citronellyl caproate of ester produced. The IR spectra of geranyl caproate as shown in Figure 2B displayed the formation of a new group at the wave number,  $\nu_{\max} = 1718.93 \text{ cm}^{-1}$ , which is stretching vibration of carbonyl (C=O) of geranyl caproate of ester produced. The stretching vibration carbonyl (C=O) of ester compounds different from the absorption of carbonyl (C=O) of caproic acid at wave number,  $\nu_{\max} = 1705.07 \text{ cm}^{-1}$  as shown in Figure 2.

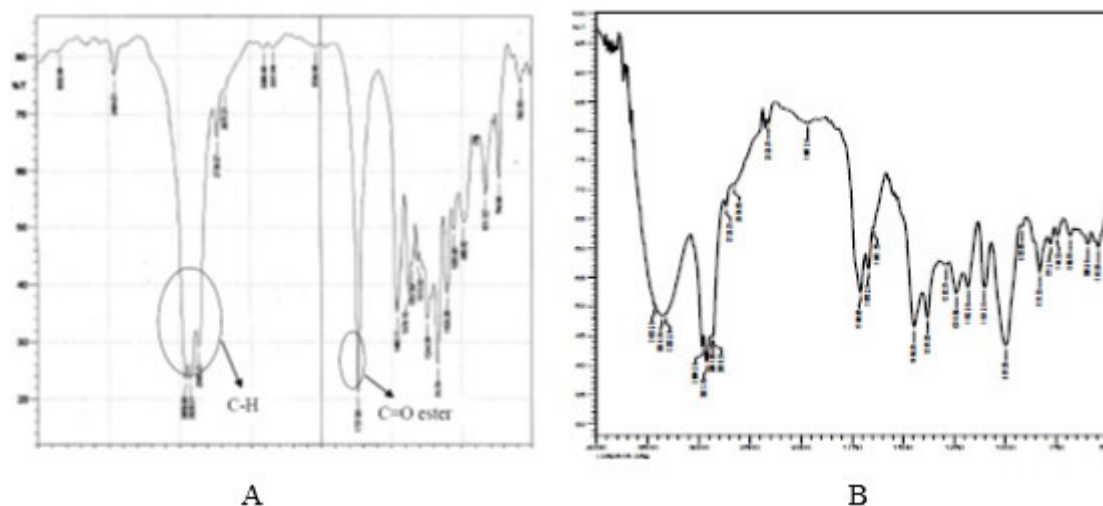
The result of GCMS analysis of citronellyl caproate showed that citronellyl caproate has been formed with indicated a dominant peak at retention time (RT) of 17.03 minutes (peak of hexanoic acid 3,7-dimethyl-6-octenyl ester, C<sub>16</sub>H<sub>30</sub>O<sub>2</sub>, MS of 254.08 mol/g). The another peaks of GCMS chromatogram indicated there were impurities and residual unreacted reactant of citronellol at retention time of 10.92 minutes (Figure 3A).

The GCMS chromatogram of geranyl caproate in Figure 3B showed that a dominant peak at retention time (RT) of 17.40 minutes (peak of hexanoic acid 3,7-dimethyl-2,6-octadienyl ester, C<sub>16</sub>H<sub>28</sub>O<sub>2</sub>, MS of 252.39 mol/g).

The <sup>1</sup>H-NMR spectra of ester compound products as shown in Table 1. The <sup>1</sup>H-NMR spectrum of citronellyl caproate data can be explained as follows: at  $\delta\text{H} = 4.08 \text{ ppm}$  (2H, t, 7.4 Hz) is a chemical shift of 2 protons (H) of the methylene (CH<sub>2</sub>) group that formed triplet as adjacent to CH<sub>2</sub> group and downfield due



**Figure 1.** The Simple Esterification Process of Caproic Acid (A) to Produce Citronellyl Caproate (B) and Geranyl Caproate (C)

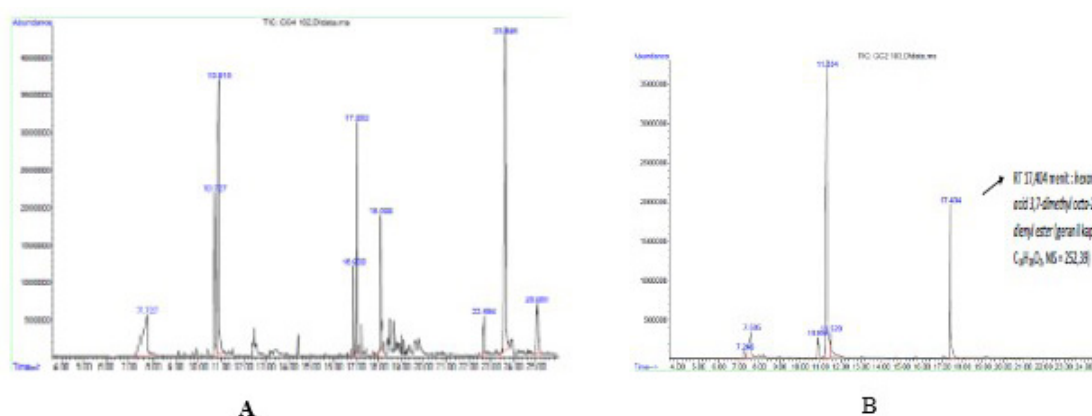


**Figure 2.** The IR spectra of citronellyl caproate (A) and geranyl caproate (B)

to interacting with electronegative of oxygen,  $\delta H = 1.60$  ppm (2H, q, 7.4 Hz) is a chemical shifts of 2 protons of  $CH_2$  group, and formed quartet as adjacent to  $CH_2$  and methine (CH) groups. The proton signal  $\delta H = 1.65$  ppm (1H, m, 7.4 Hz) is a chemical shifts of one proton of the CH group that formed multiplet as adjacent to  $CH_3$  and  $CH_2$  groups,  $\delta H = 1.58$  ppm (2H, q, 7.8 Hz) is a chemical shift of 2 proton of  $CH_2$  group that formed quartet cause interacting with 2 protons of the  $CH_2$  group and one proton of the CH group. At  $\delta H = 1.95$  ppm (2H, t, 6.7 Hz) is a chemical shift of 2 protons of the  $CH_2$  group that interact with  $CH_2$  group and  $\delta H = 5.06$  ppm (1H, t, 6.7 Hz) is a chemical shifts of one proton of the CH group that interact with 2 protons from the  $CH_2$  group, and downfield due to its double bond. At  $\delta H = 1.79$  ppm (3H, s) is a chemical shifts of methyl ( $CH_3$ ) group that interact with quaternary carbon atoms (C). At  $\delta H = 0.90$

ppm (3H, d, 7.4 Hz) is a chemical shifts of 3 protons of  $CH_3$  group that interacts with  $CH_2$  group,  $\delta H = 2.26$  ppm (2H, t, 7.8 Hz) is a chemical shifts of 2 protons of  $CH_2$  group that interacts with  $CH_2$  group. Furthermore the proton signals at  $\delta H = 1.28$ -1.66 ppm (2H, q, 7.8) is a chemical shifts of 2 protons for three  $CH_2$  groups that interacts with 2  $CH_2$  groups, and signal at  $\delta H = 0.88$  (3H, t, 6.9 Hz) is a chemical shifts of 3 proton of  $CH_3$  group that interacts with  $CH_2$  group.

The  $^1H$ -NMR spectrum of geranyl caproate data can be explained as follows: at  $\delta H = 4.13$  ppm (2H, d, 7.2 Hz) is a chemical shift of 2 protons (H) of the methylene ( $CH_2$ ) group that formed doublet as adjacent to CH group and downfield due to interacting with electronegative of oxygen and ester carbonyl (C=O) group. At  $\delta H = 5.09$  and 5.39 ppm (1H, t, 7.3 Hz) are chemical shifts of one proton of 2 CH groups that interact with 2 protons from



**Figure 3.** The GCMS chromatogram of citronellyl caproate (A) and geranyl caproate (B)

**Table 1.** The <sup>1</sup>H-NMR spectra of ester compounds

<sup>1</sup> H	Chemical shift (δ, ppm) : (δH, m, J Hz)	
	Citronellyl Caproate	Geranyl Caproate
1	-	-
2	2.26 (2, t, 7.8)	2.31 (2, t, 7,8)
3	1.66 (2, m, 7.8)	1.62 (2, t, 7,8)
4	1.28 (2, m, 7,8)	1.29 (2, m, 7,8)
5	1.30 (2, m, 7.8)	1.31 (2, m, 7,8)
6	0.88 (3, t, 6.9)	0.88 (3, t, 7,2)
1'	4.08 (2, t, 7.4)	4.13 (2, d, 7,2)
2'	1.60 (2, m, 7.4)	5.39 (1, t, 7,2)
3'	1.65 (1, m, 7.4)	-
4'	1.58 (2, m, 7.4)	2.06 (2, t, 7,2)
5'	1.95 (2, t, 6.7)	2.01 (2, dd, 7,3)
6'	5.06 (1, t, 6.7)	5.09 (2, t, 7,3)
7'	-	-
8'	1.79 (3, s)	1.67 (3, s)
9'	1.78 (3, s)	1.58 (3, s)
10'	0.90 (3, t, 7.4)	1.67 (3, s)

Note : s: singlet, d: doublet, t: triplet, m: multiplet and δ: chemical shift of spectra <sup>1</sup>H-NMR.

the CH<sub>2</sub> group, and downfield due to its double bond. At δH = 2.01 and 2.06 ppm (2H, t, 7.2 Hz) are chemical shifts of 2 protons of 2 CH<sub>2</sub> groups that interact with CH<sub>2</sub> group. At δH = 1.67 and 1.58 ppm (3H, s) are chemical shifts of 2 CH<sub>3</sub> groups that interact with 2 protons from the quaternary carbon atoms (C). At δH = 2.31 and 1.62 ppm (2H, t, 7.8 Hz) are chemical shifts of 2 protons of 2 CH<sub>2</sub> groups and formed triplet that interact with 2 protons from the CH<sub>2</sub> group. At δH = 1.29 and 1.31 ppm (2H, q, 7.8 Hz) are chemical shifts of 2 protons of 2 CH<sub>2</sub> groups that formed quartet due to interacting with 2 protons of the CH<sub>2</sub> group and one proton of the CH group, and at

δH = 0.88 ppm (3H, t, 7.2 Hz) is a chemical shifts of 3 protons of CH<sub>3</sub> group that interacts with CH<sub>2</sub> group.

#### 4. Discussion

Caproic acid ester derivative, that is citronellyl caproate (Figure 1B) and geranyl caproate (Figure 1C) with monoterpene, citronellol and geraniol has been successfully synthesized using NaOH as base catalyst activator in satisfactory yield 50.48 and 77.72%.

Identification of synthesized esters: citronellyl caproate (CC) and geranyl caproate (GC) using FTIR as shown in Figure 2 showed

**Table 2.** Calculation of ADME Properties

Compounds	Adsorption		Distribution	Toxicity Parameter (Ames Tet)		Violation of "Lipinski rule's of Five"
	HIA (%)	Caco-2 cell (nm/sec)	In-vitro plasma protein binding	Carcinogenicity Mouse	Carcinogenicity Rat	
Caproic acid	97.3862	16.6893	100.0000	Negative	Negative	No
Citronellol	97.3677	18.9366	100.0000	Negative	Negative	No
Geraniol	97.3677	23.4043	100.0000	Negative	Negative	No
Citronellyl caproate	100.0000	55.2708	100.0000	Negative	Negative	No
Geranyl caproate	100.0000	55.2233	100.0000	Negative	Negative	No

peak at 1718.39 (GC) and 1728.22 (CC) from –CO– carbonyl ester. Further analysis using GC-MS as shown in Figure 3 showed that synthesized ester compounds have base peak at  $m/z$  254.2 for citronellyl caproate and 252 for geranyl caproate mass fragment.

Caproic acid ester derivative demonstrated anticancer activity for P388 cancer cells. Microscopic observation of caproic acid and its ester derivative on P388 cell lines showed that citronellyl caproate has the best activity as the present of almost all cells unattached to the well (cells appear in irregular shape) and dead (cells appear as shrink shape with dark color). Citronellyl caproate with  $IC_{50}$   $4.15 \times 10^{-2}$   $\mu$ M showed higher activity compared to geranyl caproate with  $IC_{50}$   $11 \times 10^{-2}$   $\mu$ M and the parent caproic acid itself with  $IC_{50}$   $39.9 \times 10^{-2}$   $\mu$ M although less active than artonin E as standard material with  $IC_{50}$   $0.09 \times 10^{-2}$   $\mu$ M.

The calculations of ADME properties performed using preADMET online software<sup>12,13</sup> as shown in Table 2 revealed that caproic acid ester derivatives has HIA percentage, Caco-2 cell permeability, and in-vitro plasma protein binding slightly higher than the parent compounds, that is caproic acid, citronellol and geraniol.

Caco-2 cells are derived from colon adenocarcinoma and possess multiple drug transport cycles through the intestinal epithelium. The Caco-2 cell model is reliable in vitro model for prediction of oral drug absorption, while HIA is the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile, and feces. This calculations might explain, the adsorption and distribution of caproic acid ester derivatives are higher, so their easibility and accumulation to penetrate through cell membrane are higher. This easibility to travel through cell membrane is one of the critical factor to counteract reactive oxygen species generated by lipid peroxidations which leading to autophagy<sup>16</sup>. Drugs likeness calculations also revealed that caproic acid ester derivatives satisfies all Lipinski rule of five such as log P, hydrogen bond acceptors, hydrogen bond

donors and molecular weight and also has no carcinogenicity effect to rat or mouse.<sup>17,18</sup>

Although anticancer mechanism of caproic acid and ester its derivative is still remains unclear, our result showed that caproic acid ester derivative has potency as anticancer hence their anticancer mechanism is need to be studied further.

## 5. Conclusion

Caproic acid esters derivatives showed higher activity than their parent caproic acid for P388 cancer cell lines. ADME studies and drug likeness properties showed that the synthesized compound has no violation to Lipinski Rule of Five and has no carcinogenicity effect to rat or mouse.

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