ANTICANCER ACTIVITY OF NANOEMULSION FORMULATION OF RODENT TUBER MUTANT EXTRACT \((Typhonium flagelliforme)\) ON HUMAN BREAST CANCER CELL LINE

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ABSTRACT

Rodent tuber \((Typhonium flagelliforme)\) as an herbal medicine has a play role to against cancer disease. This study aims to identify the bioactive compounds and to evaluate the anticancer activity on MCF-7 cancer cells of rodent tuber mutant plant extract and nanoemulsion formula \textit{in vitro}. Nanoemulsion of rodent tuber mutant plant was developed with a 750 rpm homogenization technique for 30 min. The nanoemulsion were formulated with various surfactants and concentrations i.e. tween 80 (0.1%, 0.5%) and glycerol (0%, 2.5%, 5%, 10%). The best characteristic formula result was shown as F22, which has a particle size of 30.12 nm, polydispersity index of 0.51, and zeta potential of -16.20 mV. The results of the anticancer activity test have an IC\(_{50}\) value of rodent tuber mutant plant extract was 2.35 \(\mu\)g mL\(^{-1}\), and the F22 has an IC\(_{50}\) value of 1.89 \(\mu\)g mL\(^{-1}\) against MCF-7 breast cancer cells. The GC-MS analysis was showing that the extract and F22 have an anticancer compound as hexadecanoic acid (22.8%) and n-hexadecanoic acid (1.68%), respectively. The results revealed that nanoemulsion F22 is more effective at inhibiting the growth of MCF-7 cancer cells than the extract only. F22 can be used as a candidate formula for application to functional drinks.

Keywords: \textit{Typhonium flagelliforme}, Anticancer Activity, Nanoemulsion Formulation, Human Breast Cancer.

INTRODUCTION

Rodent tuber \((Typhonium flagelliforme)\) is an Indonesian herbal medicinal plant from the family Araceae.\(^4\) These plants play an important role in producing bioactive compounds against anticancer activity which use as a source of raw material for anticancer drugs. Rodent tuber has been utilized for treating cancer in some organ vitals such as lung and breast\(^22\), liver\(^21\), leukemia\(^27\), intestine, prostate gland, and cervix.\(^14\) All parts of the rodent tuber mutant plant contain anti-cancer compounds.\(^2\) The rodent tuber mutant plant has a great activity to kill breast cancer cells.\(^39\) Anti-cancer activity of rodent tuber is also seen in preventing breast, uterine cancer.\(^47\) The biological activities possessed by rodent tuber mutant plant include antibacterial, anti-oxidant effects\(^29\) toxic to \textit{Salina artemia}\(^40\) and trigger apoptosis.\(^21\) Efficacious compounds in rodent tuber mutant plants are alkaloids, saponins, steroids, and glycosides.\(^48\) The GC-MS analysis is commonly carried out to determine bioactive compounds. Gas chromatography (GC) is one of the metabolic profile analysis methods of organisms for the identification of bioactive compounds. Mass spectrometry (MS) is a method of metabolomic analysis to determine the molecular weight of bioactive compounds. This method is selective and specific. High-resolution MS can separate different compounds with similar molecular weights.\(^20\) GC-MS analysis was carried out by Mohan \textit{et al.}\(^28\) to Malaysian rodent tuber with non-polar active fractions. Sianipar \textit{et al.}\(^41,42\) have successfully analyzed the
bioactive compounds from the rodent tuber mutant clones with GC-MS. GC-MS analysis showed that superior mutant clones KB 6-1-1-2 contained six types of anticancer compounds on leaves and four types on tubers. The solid superior rodent tuber mutant has hexadecanoic acid methyl ester, squalene, octacosane, and 7-pentadecyne anticancer compounds and is not present in controls. Rodent tuber mutant plant extracts have been known to encourage apoptosis in some cancer cells *in vitro*. Rodent tuber mutant plant extracts with ethanol fraction have been proven to be effective in inhibiting the growth of T47D breast cancer cells. The extraction of rodent tubers with DCM fraction can inhibit MCF-7 breast cancer cells, inhibit cell proliferation of human T4-lymphoblastoid cancer, and can inhibit the growth of NCI-H23 cell culture of non-small cell lung carcinoma. Purnamaningsih *et al.* showed that rodent tuber mutant plant extract inhibited the proliferation of MCF-7 breast cancer cells with IC_{50} of about 7.043 μg mL^{-1}. However, the ethanol extract of rodent tuber mutant plant did not toxic and caused apoptosis to the healthy cell (CV1 normal cell line).

One of the leading causes of death in people in the world is cancer. Breast cancer is a malignant disease that causes the highest mortality rate in women. The highest prevalence in women is caused by cervical and breast cancer in Indonesia until 2017 is about 3,079 suspected breast cancer in women aged 30-50 years, while in the world it was diagnosed around 1.7 million attacking women in 2012. Cancer treatment is done through chemotherapy, but it is less effective to inhibit the growth of cancer cells because chemotherapy can cause death in healthy human cells. Therefore, the alternative treatment is using raw materials from medicinal plants, such as rodent tuber mutant plant which has anticancer potential. During this time, alternative cancer treatments are carried out by consuming juice from fresh rodent tuber plants. According to facilitate consumers, the rodent tuber plants need to be processed into functional beverage products. Functional drinks can be interpreted as beverage products that can reduce the risk of illness and optimize health condition. Functional drinks based on KB 6-1-2 rodent tuber mutant plants need to be formulated in the form of nano-emulsions so that the contents of the anticancer bioactive compounds inside can be optimally absorbed by human metabolism. Nanoemulsion technology is useful for enhancing functional food (nutraceuticals) by increasing solubility, thermal stability, bioavailability, sensory attributes, and psychological effects. Nanoemulsion has been well developed for the absorption of various drugs, including medicines, phytopharmaceuticals, diets, and nutraceuticals for the improvement of human health. Nanoemulsion is homogeneous, thermodynamically stable. Nanoemulsion can bring herbal bioactive compounds to specific target sites and maintain blood plasma concentrations for a longer period. Nanoemulsion has a role in increasing solubility, stability, permeability, and availability of herbal bioactive compounds through encapsulation. Nanoemulsion is very stable in solution. The function of nanoemulsion as great biocompatibility and stability in cytotoxicity tests. Several nanotechnology-based research results have been produced to improve bioavailability in food. According to Prakash *et al.*, said that the use of linalool nanoemulsion can be applied as a natural antibacterial and antio biofilm agent against *S. typhimurium* in the food industry. The nanoemulsion method on propolis can be used as a food preservative, preventing degradation, and covering the strong taste of propolis. This study aimed to evaluate the anticancer activity of the nanoemulsion formula of rodent tuber mutant plant extracts against MCF-7 breast cancer cells and to identify the anticancer compound content of the rodent tuber mutant plant extract and nanoemulsion formula using GC-MS method.

**EXPERIMENTAL**

**Preparation of Extract From Rodent Tuber Mutant Plant**

The rodent tuber (*Typhonium flagelliforme*) mutant plant KB 6-1-2 was harvested from the Sianipar & Purnamaningsih’s collection. The tubers were washed and dried. The powder of tubers was macerated in 96% ethanol at 24 h with a ratio of 1:3. The filtrate should be removed and filtered by using Whatman filter paper No. 1. All supernatants were evaporated to dryness under the rotary vacuum evaporator. The concentration of the crude extract was collected and utilized for the nanoemulsion formula and further analysis.

**Preparation of Nanoemulsion Formulation From Rodent Tuber Mutant Plant Extract**

Nanoemulsion was prepared the rodent tuber mutant (KB 6-1-2) plant extract (0.1% of the final emulsion) into a beaker and mixed with DMSO (0.1% of the final emulsion) until dissolved then added tween 80...
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Characterization of Particle Size, Polydispersity Index, and Zeta Potential Analysis
Two drops of nanoemulsion sample were placed into a cuvette and diluted in 2 mL water for particle size measurement. Nanoemulsion droplet size was measured using a Malvern ZetaSizer (Nano-ZS, Malvern Instruments, Malvern, UK). The particle size was measured as Z-average applying Stokes-Einstein relation with its corresponding polydispersity index (PDI). The 25 μL of the sample was placed into a capillary cell and diluted in 2 mL water to measure the zeta potential. All measurements were taken three times.

Identification of Bioactive Compounds in Extract and Nanoemulsion using GC-MS Analysis
The ethanol extract and nanoemulsion formula of rodent tuber mutant plant (KB 6-1-2) were carried out using gas chromatography-mass spectrometry (GC-MS) analysis. Extract and nanoemulsion formula 22 (F22) were injected into the GC column. 5µl volume was inserted with a 5:1 split ratio and 250°C of the injection temperature. Helium is used as carrier gas with 0.8 µl per min velocity. The column temperature was applied at 70°C with 5°C per min. The temperature reached 200°C, and it remained constant for 1 min and then will be increased at the rate of 20°C per min until the temperature reached 280°C. The mass spectrometer was operated in electron impact ionization mode with 70 eV voltage. The identification of compounds from the extract and nanoemulsion formula were analyzed by comparing the relative retention time and mass spectra with the National Institute Standard Technique (NIST) database.

In vitro Screening Test of Cancer Cell Cytotoxicity and Data Analysis
The rodent tuber mutant plant extract used for the cytotoxic activity test were KB 6-1-2 and the nanoemulsion formula 22 (F22). The MCF-7 cell line (breast cancer cells in humans) was obtained from ATCC (American Type Cell Cancer), a collection from the Division of Biology Activities at the Central Laboratory of Padjajaran University. Cytotoxic activity against breast cancer cells (MCF-7) was performed by MTT assay method. Suspension 10^3 cells (100 μL) were cultured and seeded into 96-well disk plate, and incubated at 37°C for 24 h. Medium containing rodent tuber extract (100 μL) with 1000, 500, 250, 125, 62.5 μg mL^-1 serial concentration of test solution added to each well and the disk plate was incubated at 37°C for 24 h. At the end of the incubation, the culture media containing the sample was removed and washed with 100 μL PBS (phosphate-buffered saline). For each well was added 100 μL of culture medium containing MTT and re-incubated for 4 h at 370°C. The live cells were reacted with MTT to form purple formazan. After 4 h, the stopper reagent was added to kill cells and to dissolve formazan crystals. The disk plate was plated in a shaker for 10 min then incubated at room temperature in a dark room overnight. Furthermore, the absorbance of each well was read by the ELISA reader with absorbance at a wavelength of 595 nm, and the cell proliferation inhibition (CPI) % of MCF-7 was calculated. A dose of test solution
that reduced survival by 50% (IC$_{50}$) was determined. The cytotoxic activity of rodent tuber mutant plant extract, and nanoemulsion formula 22 (F22) was assessed with IC$_{50}$ values obtained through linearity analysis between the linear concentration of the test material and the percentage of MCF-7.

**RESULTS AND DISCUSSION**

**Physical Characterization of the Nanoemulsion Formula**

Physical characteristics of the emulsion of KB 6-1-2 rodent tuber mutant plant extracts with various concentrations of surfactants tween 80 with 0.1% and 0.5% and glycerol from 0% to 10% are presented in Table-2. The nanoemulsion particle size is located in the range of 19.76 - 77.35 nm. Nanoparticles are defined as submicron (<1 μm) colloidal particles. The value of nanoemulsion polydispersity index (PDI) starts from 0.51 - 0.83. Table-2 shows that the zeta potential is negatively charged and has a value that varies from -16.34 to -23.50 mV.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Extract</th>
<th>DMSO</th>
<th>Tween 80</th>
<th>Glycerol</th>
<th>Water</th>
<th>Droplet size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F18</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>0</td>
<td>9.93</td>
<td>29.44 ± 5.26</td>
<td>0.66 ± 0.08</td>
<td>-16.34 ± 10.69</td>
</tr>
<tr>
<td>F19</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>9.97</td>
<td>25.13 ± 9.40</td>
<td>0.48 ± 0.18</td>
<td>-18.20 ± 7.92</td>
</tr>
<tr>
<td>F21</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>2.5</td>
<td>9.68</td>
<td>41.37 ± 14.98</td>
<td>0.83 ± 0.02</td>
<td>-22.20 ± 1.41</td>
</tr>
<tr>
<td>F22</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>2.5</td>
<td>9.45</td>
<td>30.12 ± 1.12</td>
<td>0.51 ± 0.22</td>
<td>-20.55 ± 4.88</td>
</tr>
<tr>
<td>F24</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>5</td>
<td>9.43</td>
<td>43.20 ± 28.79</td>
<td>0.64 ± 0.06</td>
<td>-23.50 ± 0.99</td>
</tr>
<tr>
<td>F25</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>5</td>
<td>9.47</td>
<td>19.76 ± 2.31</td>
<td>0.51 ± 0.06</td>
<td>-18.4 ± 4.38</td>
</tr>
<tr>
<td>F27</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>10</td>
<td>8.93</td>
<td>77.35 ± 52.82</td>
<td>0.82 ± 0.26</td>
<td>-22.15 ± 1.20</td>
</tr>
<tr>
<td>F28</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>10</td>
<td>8.97</td>
<td>39.38 ± 19.37</td>
<td>0.46 ± 0.23</td>
<td>-18.45 ± 2.62</td>
</tr>
</tbody>
</table>

The results showed that the emulsion of KB 6-1-2 rodent tuber mutant plant extracts was succeeded into nanoparticles at all concentrations formula. After 24 h of storage, micro-sized particles are rapidly re-aggregated where nanoemulsion with suspension forms the flocculation or sedimentation shown in the nanoemulsion solution. It can be assumed that in all concentrations of extracts and surfactants used in the production of nanoemulsions, it is still unstable. Particles formed from electrostatic reactions between extracts and surfactants are so large and dense that in the nanoemulsion formula they form aggregates into micro-sized particles.

The nanoemulsion formula 22 (F22) showed that the particle size, polydispersity index value, and zeta potential value were 30.12 nm, 0.51 and -20.55 mV, respectively. The overall results of this study can be concluded that the best formulation is formula 22 (F22) with extract and surfactant concentrations of 0.1% for tween 80 and 2.5% for glycerol. Emulsions are categorized as nanoemulsions if they have an average particle size of less than 100 nm.

The measurement results showed that the average particle size obtained by formula 22 is 30.12 nm with a PDI (polydispersity index) of 0.51. PDI is the average particle size distribution characterization as indicated by the homogenous particle size in the solution. A smaller PDI value will cause more homogeneous particle size distributions. Therefore, the F22 nanoemulsion formula obtained was homogeneous concerning the particle diameter.

According to Juniatik et al., if the zeta potential value is from 0 to ± 30 mV, this indicates the solution instability, if the zeta potential value higher than ± 30 mV indicates the stability of the solution. The formulation (F22) is not stable enough for a certain period even though it has nanoparticle size and nanoparticle size distribution. Nanoemulsions have reached aggregation after 24 h. The zeta potential of the particle will describe the repulsion force between the particles and cause the enormous zeta potential, and the dispersion system will make the nanoemulsion solution more stable. Zeta potential value is influenced by various factors, such as surfactant type, electrolyte concentration (ionic strength) dispersion media, morphology and particle size, pH of the solution, and hydration. It can be shown that the
nanoemulsion formulation is unstable for a long time even though it has nanoparticle size, but still has a proper particle size distribution.\textsuperscript{38} Harwansh \textit{et al.}\textsuperscript{12} reported that nanoparticles can increase the bioavailability of bioactive compounds through the diffusion of membranes to certain target cells. Nanoparticles are more efficient to provide pharmacological effects in smaller doses.\textsuperscript{15,50} Nanoparticles are generally advantageous because they can penetrate various spaces that cannot be penetrated by larger particles, can be made from various biocompatible materials, and can be made by simple and inexpensive methods.\textsuperscript{24}

**Analysis of Anticancer Compounds in the Nanoemulsion Formula via GC-MS**

The chemical compounds detected from F22 nanoemulsion were dimethyl sulfoxide (6.31%), glycerin (92.03%), and n-hexadecanoic acid (1.67%), presented in Table-3 and Figure-1. Chemical compounds that have been identified in rodent tuber mutant plant extracts are hexadecanoic acid ethyl ester (8.26%), hexadecanoic acid (22.8%), ethyl (9Z, 12Z) -9.12-octadecadienoate (6.67%), 9,12-octadecadienoic acid (26.13%), linoleic acid (1.41%), (9E, 12E)-9.12-octadecadienoic acid (23.4%), tricosane (1.4%), Z, Z-10,12-hexadecadien-1-ol acetate (0.83%), eicosane (1.45%), (6E, 10E, 14E, 18E) -2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (1.05%), heptacosane, 1-chloro (1.71% ), nonacosane (0.83%), Stigmasterol (1.07%) can be seen in Table 4. In the extract of rodent tuber mutant plant KB 6-1-2 shows that the 9,12-octadecadienoic acid or stearic acid is the major compound with the highest concentration which is 26.13%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>RT (%) Peak Area</th>
<th>Chemical Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoemulsion formula 22 (F22) 14,198 6,31 Dimethyl Sulfoxide 26,334 92,03 Glycerin 48,840 1.67 n-Hexadecanoic acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>RT (%) Peak Area</th>
<th>Chemical Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent tuber mutant plant extract (KB 6-1-2)</td>
<td>31,912 8,26 Hexadecanoic acid, ethyl ester</td>
<td></td>
</tr>
<tr>
<td>31,968 22,8 Hexadecanoic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32,568 1,41 9,12-octadecadienoic acid, methyl ester (linoleic acid, methyl ester/ methyl linoleate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32,954 6,67 Ethyl (9Z,12Z)-9.12-octadecadienoate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33,03 24,29 9,12-octadecadienoic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33,112 23,4 (9E,12E)-9.12-octadecadienoic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33,616 1,4 Tricosane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34,064 0,83 Z,Z-10,12-hexadecadien-1-ol acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34,147 1,84 9,12-octadecadienoic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34,505 0,56 Eicosane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35,464 0,89 Eicosane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36,263 1,05 (6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36,705 1,71 Heptacosane, 1-chloro</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38,456 0,83 Nonacosane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41,063 1,03 Stigmasterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41,152 0,04 Stigmasterol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Octadecadienoic acid or stearic acid has been reported to inhibit the development of human breast cancer cells in proliferation \textit{in vitro}\textsuperscript{49,11,5} and \textit{in vivo}\textsuperscript{5} Octadecadienoic acid or stearic acid has been shown to induce apoptosis in breast cancer cells and inhibit the breast tumor cell cycle.\textsuperscript{17,23} The n-hexadecanoic acid compound has potential and anticancer activity in cervical cancer cell lines, has cytotoxic for MOLT-4 leukemia cancer cells, and inhibits growth and induces apoptosis of cancer cells in the human stomach.\textsuperscript{39,31,51} The n-hexadecanoic acid revealed that KB 6-1-3-4 rodent tuber mutant plant extracts had shown cytotoxic activity in the MCF-7 breast cancer cell line.\textsuperscript{39}
In the comparison between the extract and F22 nanoemulsion formula, the anticancer compounds were detected only n-hexadecanoic acid or palmitic acid in F22 which have strong potential as anticancer agents. This shows that the process of F22 nanoemulsion formula causes instability or loss of some bioactive compounds that play an anticancer role. Anticancer activity in n-hexadecanoic acid as a fatty acid was proven by Helmut et al.\textsuperscript{13} to prevent diseases in breast, colon, and prostate cancer.

**Fig.-1:** The Chromatogram Graph of Nanoemulsion Formula 22 (F22) (A) and Rodent Tuber Mutant Plant Extract (KB 6-1-2) (B)

**Cytotoxic Activity of Nanoemulsion Formula and Rodent Tuber Mutant Plant Extract**

The results of the cytotoxicity test for MCF-7 breast cancer cells showed that KB 6-1-2 rodent tuber mutant plant extracts had a more significant inhibitory effect on cell growth compared to the mother plant that had IC\textsubscript{50} of 2.35 μg mL\textsuperscript{-1} within 24 h incubation. Cell viability results showed that the extract of the rodent tuber mutant plant significantly inhibited the growth of breast cancer cells that were dose and time-dependent (Fig.-2). At 24 h incubation, nanoemulsion can significantly inhibit cell proliferation. The results of this study have shown that the IC\textsubscript{50} value for F22 nanoemulsion is 1.89 μg mL\textsuperscript{-1} (Fig.-3).

From the results of the cytotoxicity test on nanoemulsion, it can be seen that the nanoparticle size of nanoemulsion makes the penetration rate of bioactive compounds more optimal than the extract. Therefore, it becomes more effective in inhibiting cell proliferation. The inhibition of cancer cell proliferation in vitro shows that nanoemulsion can penetrate the cell membrane to induce cell death. The results of this study
indicated that F22 nanoemulsion is still able to mediate the anticancer effect of breast cancer cells. This nano-size causes the surface area that is in contact with the receptor fluid to increase so that the speed of dissolving the bioactive compound is higher. The n-hexadecanoic acid compound detected from F22 nanoemulsion showed a significant IC$_{50}$ value on MCF-7 cells. This study shows that n-hexadecanoic acid is one of the bioactive compounds detected and plays an active role in inhibiting the activity of DNA topoisomerase I, which triggers the process of apoptosis and inhibits cell proliferation.

As shown in Fig.-2 and Fig.-3, there are differences in cell growth inhibitory activity between extract and nanoemulsion. F22 nanoemulsion shows that the inhibitory activity of cell growth increases with increasing concentration of serial dilution. Increasing the concentration will increase the number of bioactive compounds so that the level of toxicity increases. In the extracted sample, there was an increase in inhibition of cell growth up to a concentration of 250 μg mL$^{-1}$, which then decreased with increasing extract concentration. This phenomenon may be caused by extracts that inhibit cell growth at low concentrations but stimulate cell growth at high concentrations.

The results of physical characteristics and the content of bioactive compounds from the F22 nanoemulsion formula showed that the F22 nanoemulsion formula still has a low level of stability. However, the formula
has been characterized as nanoparticles and particle size homogeneously distributed. Hence, the ability of nanoemulsion containing anticancer compounds as n-hexadecanoic acid can penetrate well into breast cancer cell line (MCF-7). The potency to inhibit the growth of MCF-7 breast cancer cells from formula nanoemulsion (F22) is more effective than extracts. The results of F22 showed that it can be optimized to become a functional beverage formula that is useful for preventing and recovering from cancer.

CONCLUSION
The best nanoemulsion formula of rodent tuber mutant plant was carried out of formula F22. The physical characteristics of the nanoemulsion F22 have a particle size of 30.12 nm, a PDI value of 0.51 and a potential zeta value of -20.55 mV. The results of GC-MS analysis on KB 6-1-2 rodent tuber mutant plant extracts and F22 nanoemulsion have been identified as n-hexadecanoic acid or palmitic acid compounds that have the potential as anticancer. F22 nanoemulsion showed that MCF-7 cancer cell growth inhibitory activity was higher than the extract where the IC_{50} F22 value was 1.89 μg mL^{-1} and the IC_{50} value of extract was 2.35 μg mL^{-1} within 24 h of incubation. F22 nanoemulsion formula of rodent tuber mutant plant is more effective to inhibit the growth of MCF-7 cancer cells.

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