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Original Article

FORMULATION AND CYTOTOXICITY EVALUATION NANOEMULSION OF JERUJU LEAVES (*ACANTHUS ILLICIFOLIUS L*) ETHANOL EXTRACT ON T47D BREAST CANCER CELLS

HENI SETYOWATI ESTI RAHAYU, PUSPITA SEPTIE DIANITA*, KARTIKA WIJAYANTI, PERDANA PRIYA HARESMITA

[1,3]Department of Maternity Nursing, Faculty of Health Science, Universitas Muhammadiyah Magelang, Indonesia. [2,4]Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Magelang, Indonesia
*Corresponding author: Puspita Septie Dianita; *Email: puspitaseptie@unimma.ac.id

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ABSTRACT

Objective: The first cause of death in the world is cancer. The type of cancer that is often found in women is breast cancer. Various treatments and therapies have been carried out, but the incidence of cancer is increasing. This is caused by lifestyle, nutrition and environmental conditions. Cancer drugs are very toxic, expensive and resistant, so it is necessary to try other therapies that are safe, do not damage other body cells, and do not cause side effects, namely herbal or plant-based therapies. One plant that has potential as a breast cancer therapy is jeruju (*Acanthus Illicifolius L*) leaves. The aim of this research was to determine the cytotoxic effect of jeruju leaf extract (*Acanthus Illicifolius L*) on T47D breast cancer cells in a nanoemulsion preparation.

Methods: Extraction of jeruju leaves was carried out using 96% ethanol solvent, then cytotoxicity testing was carried out using the MTT test on T47D cell proliferation, then the IC_{50} value was calculated using a linear regression equation and continued with the preparation and testing of nanoemulsion preparations.

Results: Jeruju leaf extract (*Acanthus Illicifolius L*) has a strong cytotoxic activity value measured by an IC_{50} value of 1.357 µg/ml and can be packaged in a nanoemulsion preparation which has a particle size of 16.1 nm. These findings indicate that jeruju leaf extract is effective in inhibiting T47D cells *in vitro*.

Conclusion: Jeruju leaf extract (*Acanthus Illicifolius L*) has a strong cytotoxic activity value measured by an IC50 value of 1.357 µg/ml and can be packaged in a nanoemulsion preparation which has a particle size of 16.1 nm.

Keywords: *Acanthus Illicifolius L*, Breast Cancer, Nanoemulsion, T-47D cells, *In vitro*

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INTRODUCTION

The first cause of death in the world is cancer. The type of cancer that is often found in women is breast cancer. Various treatments and therapies have been carried out, but the incidence of cancer is increasing. This is caused by lifestyle, nutrition and environmental conditions. Cancer drugs are very toxic, expensive and resistant, so it is necessary to try other therapies that are safe, do not damage other body cells, and do not cause side effects, namely herbal or plant-based therapies. One plant that has potential as a breast cancer therapy is jeruju (*Acanthus Illicifolius L*) leaves. Acanthus belongs to the Acanthaceae family, which is a genus of the top group of angiospermae (flowering plants), plants that includes more than 29 species that are widely distributed in tropical and subtropical regions. *Acanthus Illicifolius l* also known as sea holly, holly mangrove, or holly-leaved acanthus, is conventionally used in Indian and Chinese medicine. It belongs to the Acanthaceae family and is a climbing plant up to 1.5 m tall. According to Indian Ayurveda, the use of *Acanthus Illicifolius l* species is useful as a nerve tonic, astringent, stimulant, and expectorant. The roots of *Acanthus Illicifolius l* are highly indicated for the treatment of cough, asthma, leucorrhea, and paralysis. The roots and leaves are indicated against animal and insect bites. The mucilage of the leaves helps in managing neuralgia and rheumatism [1]. Ecosystems of mangrove grow very widely in tropical areas, especially in the Indonesia-Pacific region. The chemical compounds of mangrove plants are rich biological applications in the field of medicine. *Acanthus Illicifoliusis l* a mangrove species that is traditionally used for various traditional human treatments. The leaves, bark, and roots of this mangrove have been used as herbal medicine for various diseases [2].

Research conducted by Rahayu, 2019 found that the leaves of jeruju (*Acanthus Illicifolius L*) contains several compounds including carbohydrates, proteins, saponins, steroids, triterpenoids, and phenols [3]. Jeruju leaves have been known to have secondary metabolites that are useful against cancer, but have been largely unexplored for their anticancer compounds and their potential in inhibiting cancer cell growth. Flavonoids can inhibit cell proliferation and induce apoptotic and *autophagic* cell death. Flavonoids also cause necrosis, cell cycle arrest, inhibit cell migration, invasion, and tumor angiogenesis. In addition, flavonoids have the ability to capture free radicals, minimize oxidative stress and control cell metabolism [4].

One of the preparations that can be used to utilize jeruju leaves is nanoemulsion preparations. Nanoemulsion is a mixture of active substances, oils, surfactants, and cosurfactants which when mixed with water will form an oil in water (O/W) nanoemulsion that is less than 500 nm in size [5]. This study aims to determine the cytotoxic effect of jeruju leaves (*Acanthus Illicifolius L*) against T47D breast cancer cells in nanoemulsion preparations that can be used as anticancer agents. The results of this study are expected to be the basis for the development and optimization of jeruju leaves (*Acanthus Illicifolius L*) as a supportive therapy for breast cancer and can be a positive contribution in the development of herbal-based breast cancer treatment.

MATERIALS AND METHODS

Materials

The samples used in this study were jeruju leaves (*Acanthus Illicifolius L*) obtained from Demang Gedi Mangrove Forest, Purworejo Regency. The materials for making extracts are 96% ethanol and distilled water. Cytotoxicity test using T-47D breast cancer cells (Parasitology Lab FKKMK UGM), RPMI-1640 medium (Gibco), MTT (Sigma), Fetal Bovine Serum (Gibco), Trypsin-EDTA (Gibco), Phosphate Buffer Saline (Sigma), trypan blue (Gibco), HCl (Sigma), Sodium Dodecyl Sulfate (Sigma), Cisplatin, pH paper universal litmus (MQuant), MicroPlate 96-Well.

Tools

The tools used were erlenmeyer tubes (Iwaki), glass beakers (Schott Durant), 1.5 ml microcentrifuge tubes (Bio-Rad), 0.2 ml tubes (Bio-Rad), tips (0.1-20, 20-200, 200 1000) µl (Bio-Rad), micro pipettes (0. 1-2, 2-20, 20-200, 200-1000) µl (Bio-Rad), phalcon tubes (15, 50) ml (Iwaki), petri dish (Petriq), refrigerator (LG), 0.02-210 g balance (AND GF-200), vortex (Gemmy Industrial), pipette pump (Bel art Alla), microcentrifuge (Sorvall), membrane filters 0.2 µm and 0.45 µm (Pall), syringe (Dong Shin GNM), laminar air flow cabinet (Gelaire ICN Biomedicals), deep freezer-80 °C (Angelantoni Scientifica), oven (Bicasa), cooling bath (Heto), ice maker (Hoshizaki), spectrophotometer (Simadzu), CO_2 incubator (Memmert), Krebs viscometer KU-2 (Brookfield), Test tube (Iwaki), Uv-Vis spectrophotometer (Aquarius), hotplate stirrer (Scilogex SCI340-4), Beaker cup (Iwaki 250 ml), Vortex (Thermolyne), Digital scale (Sartorius), Microscope (Olympus), Micropipette (Dragonlab), Yellow tip, Blue tip, Elisa microplate reader (Bio-Rad).

Research design

This research is an *in vitro* laboratory experimental research. Cytotoxicity testing applied *Post-test Only Control Group Design* at 4 triplo concentration series with positive control using Cisplatin.

Researcher phases

Sample extraction

A total of 3000 g of jeruju leaves were ground into jeruju (*Acanthus Illicifolius L*) leaf powder. Jeruju leaf powder was then macerated using 96% ethanol in a ratio of 1: 3 for 3x24 h. The maceration results were then remacerated, the maceration residue that had been obtained was then added with ethanol solvent on day 3 in a ratio of 1: 5.

Phytochemical screening test

Phytochemical screening test of jeruju leaves (*Acanthus Illicifolius L*) was an analyzed using GC-MS (Mass Gas Spectroscopy). GC-MS analysis plays an important role in the analysis of plant components whose phytochemical content is unknown [6].

Ethical clearance proposal

Ethical clereance in order to protect the research sample and researchers, ethical clearance was conducted at the Ahmad Dahlan University Research Ethics Committee Yogyakarta. This research has received a recommendation from the research ethics committee of Ahmad Dahlan University Yogyakarta with number: No. 022408110.

***In vitro* testing of jeruju (*Acanthus Illicifolius L*) leaf extract**

Preparation of cancer cell culture using T47D, preparation of cytotoxic test materials and cytotoxicity testing method MTT

Starting with preparing a 96-well plate then planting T47D cells in 100 ml of RPMI medium with a cell count of $5x10^4$ cells/well. The solution was resuspended every time to fill 12 wells so that the cells remained homogeneous. Cells were then incubated in a CO_2 incubator for 1x24 h with the aim of recovering cells after the harvesting process. Cells were treated by inverting the plate and the remaining liquid in the wells was drained using a tissue. Cells were washed using 100 µl PBS 1x before being treated with three repetitions in a concentration series in the range of 125; 250; 500; 1,000 µg/ml. Cells were then put into a CO_2 incubator for 24 h. The positive control used Cisplatin. MTT staining was performed by taking culture media containing the test compound, then washed using 100 µl PBS 1x. 100 µl MTT was added to the culture medium at a concentration of 0.5 mg/ml. Plate was put into CO_2 incubator for 4 h. Stopping the MTT reaction by adding 10% SDS (in 0.01N HCl) to the media that previously contained MTT as much as 100 µl. Furthermore, the incubation process was carried out at room temperature overnight with the plate covered with aluminum foil. To dissolve the formazan, the plate was shaken on a 100 rpm shaker, 10 min. The absorbance was read using an ELISA reader at a wavelength of 570 nm. Live cells react with MTT and produce a purple color [7].

Value calculation IC_{50}

Data analysis to determine the value of IC_{50} using Microsoft Excell 2010. Here is the calculation of the value IC_{50}:

$$\% \text{ Inhibition} = \frac{\text{Blank absorbance-sample absorbance}}{\text{Blank absorbance}} \text{ x } 100\%$$

IC_{50}

From the regression equation, y = ax+b, the value of IC_{50} = 50−ba

Value calculation IC_{50} cytotoxicity:

$$\text{Live cell percentage} = \frac{\text{Treatment absorbance} - \text{Media control absorbance}}{\text{Cell control absorbance} - \text{Media control absorbance}} \times 100\%$$

Formulation of nanoemulsion preparations

The preparation of nanoemulsion is done by optimizing the excipients used. The nanoemulsion formulations used consist of Formulation 1 (F1), Formulation 2 (F2), Formulation 3 (F3) and Formulation 4 (F4), where each formula uses a different concentration of emulsifier [8].

Table 1: Nanoemulsion formulation of jeruju leaf extract

Composition	Formula			
	F1	F2	F3	F4
Mangrove leaf extract	1.5g (2,5%)	3g (5%)	1.5g (2,5%)	3g (5%)
Lecithin	3g (5%)	1,5g (2,5%)	1,5g (2,5%)	3g (5%)
Tween-80	18 ml (30%)	18 ml (30%)	18 ml (30%)	18 ml (30%)
Aquadest	62,5 %	62,5 %	65 %	60 %

The nanoemulsion was made by mixing jeruju leaves with excipients using ultra turrax to break the particle size into smaller ones. Furthermore, *In Proccess Control* testing of the preparation was carried out to see the most optimal preparation of the four formulas, as well as particle size testing.

Testing the physical properties of nanoemulsion preparations

Testing the physical properties of nanoemulsion preparations aims to determine the character of the nanoemulsion produced and adjusted to the parameters that have been set. Testing the physical properties of nanoemulsion preparations include:

a. pH test, carried out using a pH meter. The required pH is in the range of 6-8

b. Viscosity test, aims to determine the viscosity of the preparation that has been produced in order to facilitate the pouring process when it will be used.

Centrifugation test, carried out to see whether or not separation occurs in the resulting preparation. The centrifugation test was carried out using a centrifugator with a speed of 400 rpm which was carried out for 30 min.

RESULTS AND DISCUSSION

Phytochemical screening test

Ethanol fraction of jeruju leaves using GCMS analysis was carried out by as much as 1 µl of hexane fraction from ethanol extract of jeruju leaves (*Acanthus Illicifolius L*) was used in GC-MS for analysis of various compounds. A sample of 1 µl was injected into the GC-MS, then the column used was the agilent capillary model number 19091S-433HP-5MS5%PhenylMethylSiloxane with a length of 30 m, a diameter of 250 µm, and a thickness of 0.25 µm. The oven temperature used was between 100-220 °C. The

temperature increase rate was 15 °C/min, and the lowest rate was 1.0 ml/min. The carrier gas was helium under pressure of 10.5 psi, and the total rate was 140 ml/min, and the separation ratio was 1:50. The components being evaluated will be detected in a mass detector. The spectrum components of known compounds will be stored in the NIST library and determined based on the compound name, molecular weight, and belonging to the compound group such as triterpenoids, alkaloids, flavonoids, lermic acids, phenols, and other compounds that are useful compounds for GC-MS analysis [3].

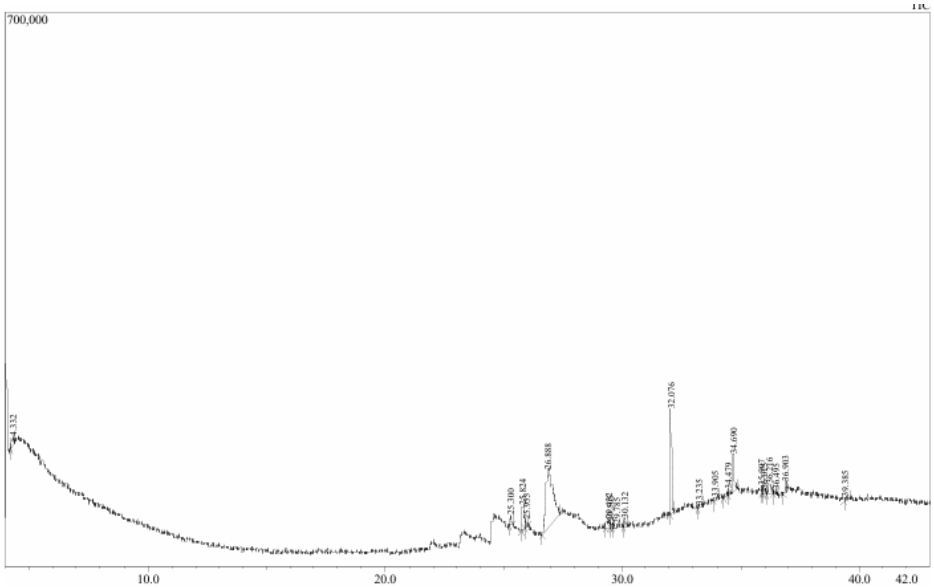

Fig. 1: GCMS of ethyl acetate fraction of jeruju (Acanthus Illicifolius L) leaves, based on the results in fig. 1, it can be seen that the ethyl acetate fraction of jeruju leaves table 2

Table 2: Profile bioactive compound of ethyl acetate fraction of jeruju (*Acanthus Illicifolius L*) leaves

Compound name	Peak area (%)	Function	Referance
Methane, sulfinylbis-(CAS)	1.87	-	
13-Hexyloxacyclotridec-10-en-2-one	1.54	Antifungal	[9]
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(CAS)	5.77	-	
2-Methyl-2-[(1E,3E,5E)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexenyl)-1,3,5-hexatrienyl]-1,3-dioxolane	1.53	-	
9-Octadecenoic acid (Z)-(CAS)	49.85	Antimicrobial, anti-inflammatory, and antioxidant effects.	[10]
propyl 10-undecenoate	2.11		
OXACYCLOPENTADECAN-2-ONE, 15-METHYL-	1.00		
3-(Benzyloxy)-3-methylheptanoic acid	1.89	-	
1,2-Benzenedicarboxylic acid, dioctyl ester (CAS)	1.71	-	
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-(CAS)	10.75	Antioksidan, antitumory, and immunostimulan.	[10]
3-(5-Cyano-4-methoxycarbonylmethyl-4,5-dimethyl-2-thioxo-pyrrolidin-3-yl)-propionic acid, methyl ester	1.34	Antioxidant and antimicrobial agent	[11]
Spirost-8-en-11-one, 3-hydroxy-, (3. beta.,5. alpha.,14. beta.,20. beta.,22. beta.,25R)-(CAS)	1.72	Antibacterial, Anti-Biofilm, Antioxidative, Antidiabetic,	[12, 13]
12-Methylene-11,12b,14b-trimethyl-3,4-dihydroxy-pentacyclodocosane	1.17	-	
alpha.-Tocopherol-. beta.-D-mannoside	7.62	Antioxidant, antimicrobial, anticancerous and enzyme inhibitory	[14]
Cyclohexene, 1-pentyl-4-(4-propylcyclohexyl)-	0.97	-	
1,2-Bis(trimethylsilyl)-3-methylbenzene	0.98	-	
Stigmasta-5,22-dien-3-ol, (3. beta.,22E)-(CAS)	3.63	Anti-diabetic	[15]
1-bromo-3-(epoxymethylene)-4-[(t-butyl)dimethylsilyloxy]butane	0.96	-	
Stigmast-5-en-3-ol, (3. beta.)-(CAS)	2.51	Anti-diabetic	[15]
3-(Cis-2'-Hydroxy-cyclohexyl)propanol	1.08	-	

Based on the results of the GCMS test, the compound 9-Octadecenoic acid (Z) is the most dominant compound with an area percentage of 49.85%. The compound 9-Octadecenoic acid (Z) has biological activity as other biologically active substances, namely supraena which has anesthetic activity [16]. Low percentages of 9-Octadecenoic acid (Z)-, methyl ester and 9, 12-Octadecadienoic acid methyl ester (E,E) were identified in the crude extracts of B. hispida and C. mochata. These compounds have great antioxidant, anticancer, and anti-inflammatory properties.

In vitro testing of jeruju (*Acanthus Illicifolius L*) leaf extract

One of the most commonly used cytotoxicity test methods *in vitro* is the MTT test method. The MTT test is a coorimetry test used to measure the metabolic activity of living cells which is carried out by

adding samples to each well and then incubating, where during incubation MTT will be yellow which means the ability of *nicotinamide adenine dinucleotide phosphate* (NADPH)-dependent cellular oxidoreductase enzymes. The cytotoxicity test uses jeruju leaf extract as the active substance and Cisplatin as a positive control which will then be tested against T47D cells. Cisplatin is a platinum-based chemotherapy drug used for cancer therapy, such as ovarian, testicular, and bladder cancer. Cisplatin is one of the most widely used anticancer drugs due to its wide efficacy for therapy of various types of cancer. The working principle of Cisplatin in fighting cancer is by causing a cytotoxic effect on cancer cells. Cisplatin interacts with DNA to disrupt DNA transcription and cause cell apoptosis. Each concentration of jeruju leaf extract and Cisplatin is then calculated for its IC_{50} value to determine the inhibitory activity or ploriferation of T47D cells. IC_{50} *(Inhibition Concentration 50%)* value is a measure of the concentration of drugs or compounds needed to inhibit certain biological or biochemical processes by 50%. The IC_{50} value of Cisplatin is 1.343 µg/ml, the IC_{50} value of jeruju leaf extract at a concentration of 200 g is 1.391 µg/ml, and the IC_{50} value of jeruju leaf extract at a concentration of 400 g is 1.357 µg/ml. This shows that jeruju leaf extract has strong cytotoxic activity in inhibiting T47D cells [17]. The results of the IC_{50} value can be seen in fig. 1,2,3.

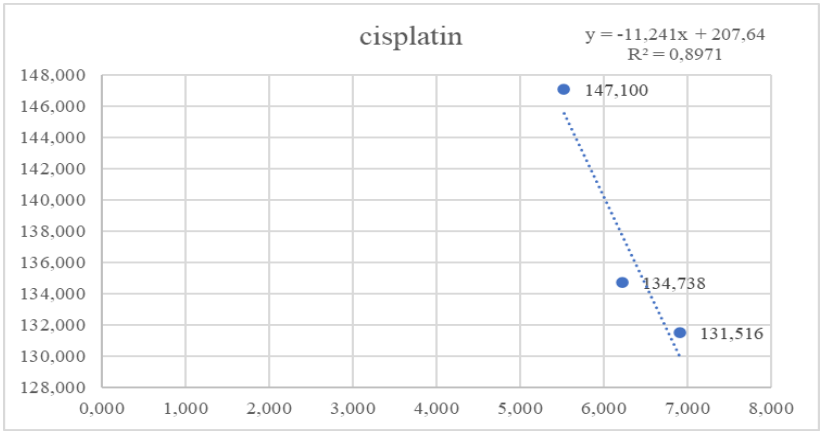

Fig. 2: IC_{50} value graph of cisplatin

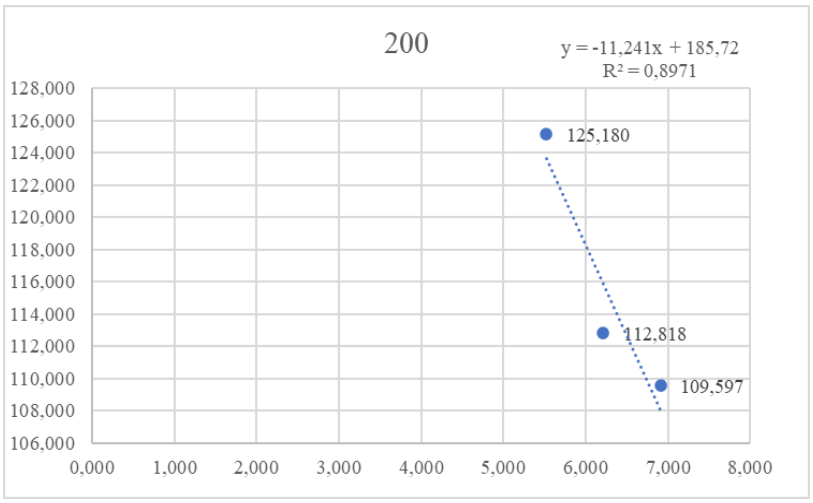

Fig. 3: IC_{50} value graph 200 g extract

To determine the cytotoxic effect of the isolate, it is necessary to calculate the IC_{50} of the percentage of inhibition. The IC_{50} value is the concentration of a compound that causes inhibition of life in 50% of the cells. This process usually involves constructing a dose-response graph and interpolating IC_{50} values from that graph or through calculations using a regression equation. The linear regression equation for Cisplatin is Y =-11,241x+207,64 with R^2 = 0,8971. While the linear regression equation for concentration extract 200 is Y =-11,241x+185.72 with R2 = 0.8971, and linear regression equation for concentration extract 400 is Y =-8.9178x+193.21 with R2 = 0.7953. The results in fig. 1 show the IC_{50} value in positive controls and extracts. The positive control using Cisplatin produced an IC_{50} value of 1.343 µg/ml, the IC_{50} value of jeruju leaf extract at a concentration of 200 g was 1.391 µg/ml, and the IC_{50} value of jeruju leaf extract at a concentration of 400 g was 1.357 µg/ml. This shows that jeruju leaf extract has strong cytotoxic activity in inhibiting T47D cells. Each treatment, namely positive control, 200 g of jeruju leaf extract and 400 g of jeruju leaf extract, was diluted triplo at 250, 500 and 1000 ppm dilutions. The quantitative cytotoxicity test requires analysis of the calculation of the IC_{50} value, where the IC_{50} value shows the concentration needed to inhibit the growth of T47D cancer cells by 50% of the total population. The smaller the IC_{50} value, the greater the cytotoxic activity. The determination of the IC_{50} value is calculated based on the linear regression between the concentration of jeruju leaf extract and the percent of cell viability. According to Kuete *et al.* [18], IC_{50} values<50 µg/ml are categorized as having a strong cytotoxic effect, if the value of 50-<200 µg/ml is categorized as moderate cytotoxic, and the value of 200 µg/ml-<1000 µg/ml is categorized as having a weak cytotoxic effect and IC_{50} values>1000 µg/ml have no cytotoxic effect [19]. Based on the results of the IC_{50} value study, it shows that jeruju leaf extract provides a cytotoxic effect with strong activity. Jeruju leaves have potential as a source of bioactive metabolite components, with chemical compositions such as carbohydrates, proteins, saponins,

alkaloids, flavonoids, fatty acids, steroids, lignans and phenol components, terpenoids [20] and megastigmane glycosides and have pharmacological activities including anticancer [21]. Jeruju leaves contain flavonoid compounds that can be used for cancer treatment. Flavonoids can inhibit cell proliferation and induce apoptotic and *autophagic* cell death. Flavonoids also cause necrosis, cell cycle arrest, inhibit cell migration, invasion, and tumor angiogenesis. In addition, flavonoids have the ability to capture free radicals, minimize oxidative stress and control cell metabolism [4]. Some theories report that some classes of compounds are efficacious as anti-cancer such as flavonoids and saponins. Flavonoids can be anti-cancer through the mechanism of activating the apoptotic pathway of cancer cells through the process of fragmentation of DNA. This fragmentation occurs through the release of DNA proximal chains by radical compounds such as hydroxyl radicals. In addition, flavonoids also work by inhibiting protein kinase activity so that the signal transduction pathway from the membrane to the cell nucleus will be inhibited [22]. In addition, tannin compounds have anticancer effects, with the mechanism of activating the cancer cell apoptosis pathway due to DNA fragmentation. Saponin compounds are also known to have anticancer potential by inhibiting the formation of overexpressed Bcl-2, inducing underexpressed caspase-3 protein, increasing p53 expression, and can also trigger G1 cellcycle arrest.

Formulation of nanoemulsion preparations

Jeruju leaves (*Acanthus Illicifolius*) have a strong cytotoxic effect, so they can be formulated into nanoemulsion preparations that can make it easier for people to consume jeruju leaves. Nanoparticles are one of the steps or solutions to particle size that aims to increase the bioavailability of active herbal compounds. Nanoparticles are solid colloidal particles with a diameter of 1–1000 nm. Nanotechnology is one of the most modern and significant technological sciences used in various technologies. One of the impacts of nanotechnology has been clearly visible in recent years. Nanoparticles are made in various ways, including chemical, physical, and biological approaches with biological methods that are increasingly prominent because they are environmentally friendly, low cost, and safe; this method is also known as green nanoparticle synthesis. Research on nanotechnology is currently growing rapidly because it can be applied widely, one of which is in the health sector [23]. Of the four formulas used in the study, the resulting preparations are listed in table 2 below.

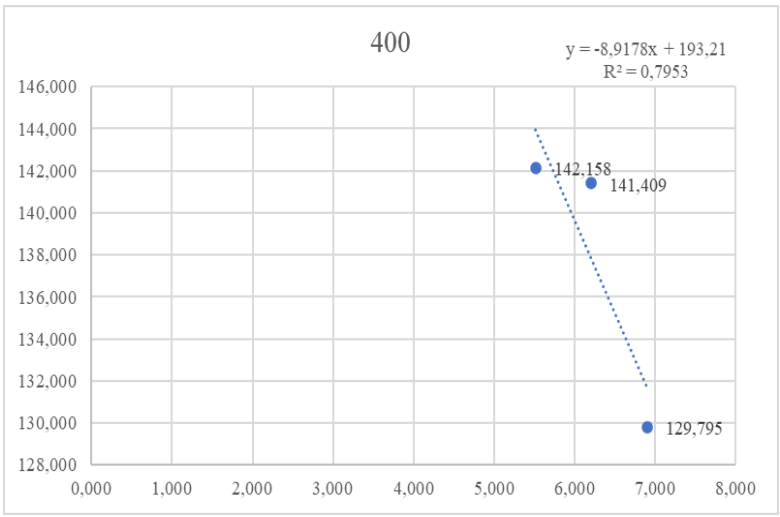

Fig. 4: IC_{50} value graph 400 g extract

Table 3: Results IPC (In proccess controll) nanoemulsion of jeruju leaf extract (Acanthus illicifolius L)

Testing	Formula 1	Formula 2	Formula 3	Formula 4
Organoleptis	Green and smells typical of jeruju	Green and smells typical of jeruju	Green and smells typical of jeruju	Green and smells typical of jeruju
pH	7	7	7	7
Emulsion stability (centrifugation method)	No separation, settling and turbidity	No separation, settling and turbidity	No separation, settling and turbidity	No separation, settling and turbidity
Percent transmittance	92,3%	81,8%	91,0%	90,6%
Viscosity	1035	584	102	982

The organoleptic test results of nanoemulsion preparations of jeruju leaf extract *(Acanthus Illicifolius L)* showed that the four nanoemulsion formulas were green in color and had a distinctive smell of jeruju leaves. The organoleptical test results can be seen in fig. 4 below.

Fig. 4: Organoleptical test of nanoemulsion of jeruju leaf extract (*Acanthus Illicifolius L)*

All three formulas produced a pH of 7, which indicates that the formulation of jeruju leaf extract (*Acanthus Illicifolius L)* preparation is included in the neutral pH. This proves that the nanoemulsion preparation of jeruju leaf extract *(Acanthus Illicifolius L*) is safe to use. Preparations that are too acidic can irritate the stomach, while preparations that are too alkaline can be corrosive to the gastric mucosa [24].

Viscosity determination was carried out to determine the level of viscosity of the jeruju leaf extract *(Acanthus Illicifolius L*) nanoemulsion preparation produced. Viscosity is one of the test parameters for the physical properties of emulsions because the higher the viscosity value of the emulsion, the speed of separation of the emulsion will decrease so that it can cause creaming, namely the separation of the dispersed phase which forms a layer above the surface of the continuous phase [25]. Nanoemulsion is an emulsion preparation with droplet sizes ranging from 10 to 1000 nm. Nanoemulsions generally consist of oil, water, and surfactants. The selection of surfactants is very important because the surfactant aims to form and stabilize nanoemulsions, where Nanoemulsions are not thermodynamically stable, but kinetically stable. This is because the separation of the nanoemulsion phase occurs if given enough time. Nanoemulsions have been developed for various applications in pharmacy, where the development of nanoemulsions must be biocompatible without any toxic effects. Based on this, the selection of oil and surfactants is important. Biocompatible oils and surfactants are desired, such as vegetable oil or pharmaceutical grade oil. Proteins and lipids have also been widely used as surfactants to stabilize nanoemulsions [26].

Table 4: Particle size analyzer data

Replication	1	2	3
Droplet size (nm)	13,8	16,1	18,3
PDI	0,441	0,483	0,548
Zeta Potensial (mV)	-26,2	-26,9	-27,5

The level of clarity of a nanoemulsion can be determined by the transmittance percentage test. The transmittance percentage results of formula 1 are higher than the other formulas and are close to 100. A high transmittance percentage value indicates that the particle size in the preparation is getting smaller. Preparations that have very small particle sizes when passed by light, the light beam will be transmitted, so that the color of the solution in the preparation will look transparent and the transmittance percentage value will be greater [27]. Based on the transmittance percentage results, the next step is nanoemulsion characterization. Characterization of nanoemulsion was carried out using Particle Size Analyzer (PSA) to determine the particle size of nanoemulsion produced. Nanoparticles were characterized based on percent transmittance, particle size, polydispersity index (PI), zeta potential, encapsulation efficiency, and morphology. Nanoemulsion of jeruju leaf extract was loaded into PSA to measure its particle size and polydispersity at 25 °C and 90° scattering angle [28]. Nanoparticles are a broad spectrum of materials containing special compounds that will be characterized by particle sizes smaller than 100 nm. Particle size is greatly influenced by the physical and chemical properties of the material to be used. Nanoparticles can be synthesized chemically or biologically, where the chemically produced procedure has many negative impacts because some harmful substances are absorbed on the surface [29].

The expected droplet size in nano form is around 20-100 nm [30]. The average droplet size can be seen in fig. 5.

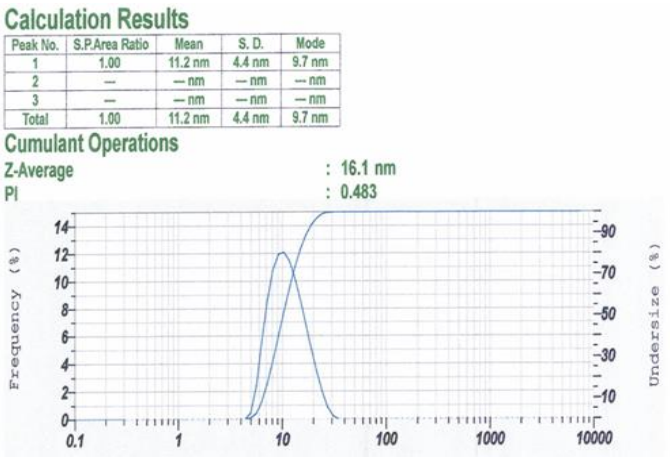

Fig. 5: Characterization of jeruju leaf extract nanoemulsion

The particle size results from fig. 3 show that the nanoemulsion preparation of jeruju leaf extract has a measurement test obtained a value of 16.1 nm. The magnitude of the particle size is less than 200 nm which has the positive side of being able to pass through the galvanic cell more easily. This plays an important role in the absorption and distribution of active substances. The PDI test showed a result of 0.483. That is, the dispersion is high because the PDI value corresponds to the particle size distribution in the sample. Meanwhile, PDI values that are smaller or closer to 0 or between 0.05-0.7 are considered to have uniformity. Potential zeta test value obtained-26.9 (stable charge). In other words, the level of stability is relatively high with the electrostatic type. Whereas in states that the potential zeta test number is classified as sterically stable if it shows more than+20 mV and less than-20 mV [31]. Nanoemulsion preparations are preparations consisting of a mixture of oil and water that are finely dispersed and stable, with a droplet size of less than 100 nm. Some of the characteristics possessed by nanoemulsion preparations, including nanoemulsion preparations that are more thermodynamically stable, transparent, clearer, good physical stability, and nontoxic. Nanoemulsion is also one of the most efficient and thermodynamically stable nanoterdispersion systems due to the transparent or translucent dispersion of oil and water stabilized by an interfacial layer of surfactant and cosurfactant molecules that have droplets less than 100 nm in size [30]. Nanoemulsions have unique properties such as small droplet size, high stability,

transparent shape and good rheology. These properties make researchers interested in developing nanoemulsion preparations in the food, cosmetics and pharmaceutical industries, because they have excellent drug absorption rates [32].

Nanoemulsion is greatly influenced by temperature, the composition of the materials used, surfactants, and the inversion point which will determine the number of droplets produced [33]**.** Nanoemulsions are currently being developed in the pharmaceutical industry. Nanoemulsion formulation has several advantages, namely accelerating drug delivery to target cells and for diagnostic purposes. The most important advantage of nanoemulsion preparations is that they can cover the unpleasant taste of the emulsion and can protect the preparation from oxidation [34]. The current development of nanoemulsion preparations is the manufacture of nanoemulsions for cancer treatment. One chemotherapy agent that can be made in a nanoemulsion preparation is jeruju leaves which are derived from herbal ingredients that have high bioavailability which can later be used as an alternative herbal or natural-based breast cancer treatment.

CONCLUSION

Jeruju leaf extract (*Acanthus Illicifolius L*) has a strong cytotoxic activity value measured by an IC50 value of 1.357 µg/ml and can be packaged in a nanoemulsion preparation which has a particle size of 16.1 nm. These findings indicate that jeruju leaf extract is effective in inhibiting T47D cells *in vitro*.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

We have noconflicts of interest to disclose.

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