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Telah melaksanakan penelitian dengan judul sebagai berikut :

No.	Judul Karya Ilmiah	Tahun Pelaksanaan Penelitian
1.	Phylogenetic Analysis and Antibiotics Resistance of Listeria Monocytogenes Contaminating Chicken Meat in Surabaya, Indonesia	2020
2.	Detection of bla TEM Gene of Klebsiella pneumoniae Isolated From Swab of Food-Producing Animals in East Java	2018
3.	Perbandingan Sekuens Nukleotid gZP3 Terhadap Sekuen Konsensus Nukleotid ZP3 Wanita dan Beberapa Spesies Untuk Pengembangan Bahan Imunokontrasepsi	2011
4.	Potentials of Nggorang Leaf Standard Extract (Salvia Ccidentalis) Study: Ethnopharmacology, Standardization, Antioxoxide, Sitotoxicity and Working Mechanisms	2019

Adapun penelitian tersebut tidak perlu dilakukan Uji Etical Clearence karena tidak menggunakan hewan coba.

Demikian Surat Keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar**

Surabaya, 13 Februari 2023
Wakil Dekan III,

Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH
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Potentials of Nggorang Leaf Standard Extract (Salvia Ccidentalis) Study: Ethnopharmacology, Standardization, Antioxoxide, Sitotoxicity and Working Mechanisms

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Abstract

The use of medicinal plants is used as the main therapy as well as additional therapy to enhance one's immunity or maintain health and fitness. Nggorang Leaf Extract (EDG) polyphenol and flavonoid compounds and essential oils. Flavonoids are powerful antioxidants that work as a cancer prevention and also have antimicrobial effects. This study aims to prove the potential of EDG as an antioxidant and antiproliferation of Hela cancer cells. This research includes *Quasy experiment* research The conclusion of this study shows that Nggorang Leaf Extract (EDG) has potential as an antioxidant against DPPH radicals at IC50 = 34,407 ppm; Nggorang Leaf Extract (EDG) has potential as an antiproliferation of T47D IC50 cancer cells = 201 ppm; Nggorang Leaf Extract (EDG) has potential as an antiproliferation of Hela IC50 cancer cells = 187 ppm; Nggorang Leaf Extract (EDG) has the potential to reduce ROS activity in T47D cancer cells by 54.34% at a concentration of 50 ppm; Nggorang Leaf Extract (EDG) has the potential to reduce ROS activity in Hela cancer cells by 58.33% at a concentration of 50 ppm; Nggorang Leaf Extract (EDG) has the potential to increase P53 gene expression in T47D cancer cells by 94.13% at a concentration of 50 ppm; Nggorang Leaf Extract (EDG) has the potential to increase P53 gene expression in Hela cancer cells by 92.05% at a concentration of 50 ppm. The novelty of this study is that Nggorang leaves (*Salvia occidentalis)* can be used as a preventative for breast cancer and cervical cancer.

Keywords: *Salvia Occidentalis, Ethnopharmacology.*

Introduction

The tendency of *back to nature* today, causing the use of herbal medicines become popular not only among rural communities but also urban communities. herbs are also popular in the community based on the information of herbal products in pharmaceutical dosage forms. *World Health Organization* estimates that 80% of the world's population still entrusts health with traditional medicine, especially those sourced from medicinal plants.

Research on the efficacy of herbal medicine has long been done, but until now there are still many aspects of herbal medicine that have not been disclosed by researchers. One of the uses of herbal medicines that are being developed is to help cure cancer [2] One of the plants used by the Manggarai community is nggorang leaf (*Salvia occidentalis)*. This plant is commonly found in Tenda Village, when harvesting leaves are one year old and used as medicine. Preventive efforts for these leaves are hereditary used as an anticancer (7 leaves boiled with 200 ml of water until ½ then drunk), for wounded breast cancer, stamina enhancer, cough, influenza, hemorrhoids[3].

Preliminary studies on Nggorang Leaf Extract (EDG), have been proven to have strong antioxidants and potential as cervical anticancer (hela cells); potential as an antibacterial *Candida albicans* has a inhibitory power of 12, exceeding positive control (Nystatin) [4]. Polyphenols are also antioxidants, which inhibit enzymes that can stimulate the growth of cancer cells and suppress the immune response and help slow the development of tumors [1]

Although it has been used empirically by the Manggarai community for anticancer treatment, scientific proof does not yet exist. So that nggorang leaves (*Salvia occidentalis)* are very potential to be

researched and developed considering that cancer cases in Indonesia are still very high.

Research Location and Time: Extraction process and standardization will be carried out in the Laboratory of Polytechnic ice MoH Makassar and Biofarmaka Laboratory Faculty of Pharmacy UNHAS, Anatomy Parasitology and Pathology Section of the Faculty of Medicine UGM. Research time 2014-2015.

Materials and Research Tools: The research materials used were (1) nggorang leaf extract, (2) material for standardization of extract (3) materials for cytotoxicity activity(4) materials for cytotoxicity activity apoptosis and cell cycle analysis: *flow charts reagent* and kit PI/RNase *staining buffer* (5) materials for cytotoxicity activity analysis of P53 and ROSprotein expression. The tools needed in this research tool maceration, rotary evaporator, freeze dr yer, UV-Vis spectrophotometer, *atomic absorption chromatography* (AAS), high performance liquid chromatography (HPLC), column C18, plate TLC F254, scales analiti k incubator CO_2, inverted microscope,

Data Collection Procedure

1. **Antioxidant Activity Test d ith Radical DPPH:** Preparation of 0.4 mM DPPH Solution: A DPPH 0.4 mM solution was prepared by dissolving as much as 15.7 mg DPPH with ethanol 96% pa to 100 mL.
2. **Anti-oxidant activity test of EDG extract with free radical DPPH:** Antioxidant activity test of ethanol extract of leaves of Nggorang by DPPH method was carried out in accordance with the method carried out by Hua with a slight modification (Hua, 2014). A total of 1 mL decocta and ethanol extract of leaves of people with a concentration of 50 µg/mL, 75 µg/mL, 100 µg/mL, 125 µg/mL and 150 µg/mL were added to 1 mL DPPH 0.4 mM and sufficient volume with ethanol 96% pa to 5 mL. The mixture is then shaken and incubated in a room temperature for 30 minutes in a dark place. This solution was then measured for absorbance at λ516 nm. The same treatment was also carried out for blank solutions (DPPH solutions that did not contain test material) and positive control of vitamin C with concentrations of 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, and 6 µg/mL. The absorbance measurement data was analyzed by the percentage of antioxidant activity using the following equation:
3. **Cytotoxicity Activity Test:**
1. **Making Culture Medium:** The culture medium used in this study was the RPMI 1640 medium for T47D, Hela cancer cell culture while the Vero normal cells used M199 medium.
2. **Creation of a Growing Medium:** Growing medium of RPMI 1640 and M199 was prepared by mixing 20 0 mL medium solution with 20 ml BSA, 4 mL penicillin-streptomycin and 1 mL fungizone.
3. **Making MTT Solution 5 mg/mL:** MTT solution 5 mg/mL was prepared by dissolving 50 mg MTT with PBS pH 7.4 to 10 mL.
4. **Manufacture of PBS solution pH 7.4:** A total of 80 g NaCl, 0.2 g KH_2PO_4, 115 g Na_2HPO_4 and 0.2 KCl were dissolved to 900 mL with aquadest. The solution is then adjusted to pH by adding 0.1 N HCl solution or 0.1 N NaOH to 7.4 and then the volume is sufficient to 1,000 mL with distilled water. The solution was then sterilized with an *autoclave* at 121 ^{0}C for 30 minutes.
5. **Making SDS 10%:** A total of 10 g of SDS was dissolved with distilled water up to 100 mL HCl 0.01 N.
6. **Thawing cells:** Cancer cells T47D, HeLa and normal cells Vero obtained from the Department of Parasitology Faculty of Medicine. All mediums were given a 10% BSA supplement, penicillin-streptomycin 1% and fungizone 0.5%. Cells in the *flash culture disk were* incubated at 37 ^{0}C with 5% CO_2 gas flow. Cell proliferation was observed under an inverted microscope and confluent cells (80-90%) were harvested.
7. **Cell Harvesting:** The medium is discarded, then the *flash culture disk is* washed with PBS pH 7.4. Then trypsin-EDTA 0.5% to taste and then incubate in a 37 ° C incubator with a flow of 5% CO_2 for 1-3 minutes. Add enough growth medium and then poured into a sterile *conical tube* then centrifuged for 5 minutes at 4 ° C. Then count the number of cancer cells with a *haemocytometer.* Cell suspension was added by a number of growing medium to obtain a cell concentration of 1 x 10^4 cells/100 µL medium and cells were ready for use for testing. Sample Testing with MTT Method **assay**

4. **Examinationof P53 Protein Expression (KIT):** Analysis of P53 protein expression was performed by the immunohistochemical (IHC) technique.

Data analysis: Percentage inhibition data obtained in the antioxidant test and the subsequent cytotoxicity test were calculated by IC $_{50}$ by probit analysis using SPSS software. Data on percent cell accumulation at each phase of the cell cycle, percent apoptosis, percent P53 protein expression were tested for normality with the *Shapiro-Wilk test* and homogeneity with the *Levene test.* If the data is normal and homogeneous distributed, then *Anova one way* statistical analysis will be performed. If the data is not homogeneously distributed then proceed with the Anova parametric test and continue with the Mann Whitney test.

Results and Discussion

Research result

1. **Effects of EDG on Hela Cell Viability:** Observation of the effect of EDG administration on Hela cell viability was carried out using the *ELISA* method.

Table 1 : Results of Testing the Effect of Giving Nggorang Leaf Extract on Hela Cell Viability After 24-hour Incubation

Concentration	Viability	Elementary school
15,625	114,2693	0.1418
31.25	117.9399	0.1004
62.5	129.6398	0.1962
125	109.9105	0.0830
250	93,2095	0.1980
500	5.2994	0.0221
1000	5.2306	0.0161

Table 2: Test Results of the Effect of Giving Nggorang Leaf Extract on Hela Cell Viability After 48 hours Incubation

Concentration	Viability	Elementary school
15,625	151.9650	0.1689
31.25	133.6540	0.1080
62.5	131,4321	0.1719
125	91,249	0.2320
250	18.3675	0.0799
500	16.3529	0.1286
1000	8.5016	0.0255

Table 3: Test Results of the Effect of Giving Nggorang Leaf Extract on Hela Cell Viability After Incubation in 72 hours

Concentration	VIABILITY	Elementary school
15,625	114,9654	0.0639
31.25	102.3924	0.0093
62.5	90.9399	0.0044
125	45,337	0.0026
250	12.44747	0.0038
500	12.1725	0.0060
1000	11.6029	0.0020

Based on tables 5.8 - 5.10 and figures 5.7 - 5.9, various IC $_{50}$ values are obtained. The data shows that the incubation time of EDG in hela cell culture gave different results of viability and IC $_{50}$ values. The longer the incubation period, the smaller the IC $_{50}$ concentration used to kill cells. The EDG concentration needed to produce 50% activity after 24-hour incubation obtained IC $_{50}$ values 523.7039 ppm ; after 48 hours incubation obtained Value IC $_{50}$ 327.00 ppm ; after 72 hours of incubation obtained Value IC $_{50}$ 187,000 ppm

2. **Effect of Doxorubin on Hela Cell Viability:** Testing of doxorubin drug activity on cell viability was carried out using the *ELISA* method.

Table 4: Test Results of the Effect of Doxorubin Administration on Hela Cell Viability After 24-hour Incubation

Concentration	Viability	Elementary school
1. 5625	110.0934	0.0119
3. 125	109,1381	0.0040
6. 25	100.6793	0.0060
12. 5	80.5322	0.0090
25	68.7199	0.0095
50	30.4290	0.0026
100	5.9870	0.0026

Table 5: Test Results of the Effect of Doxorubin Administration on Hela Cell Viability After 48 hours of Incubation

Concentration	Viability	Elementary school
1. 5625	26.7947	0.0472
3. 125	20.1550	0.0038
6. 25	16,3802	0.0090
12. 5	11.3583	0.0025
25	5.2578	0.0010
50	1.4493	0.0029
100	1.2471	0,0006

Table 6: Test Results of the Effects of Doxorubin Administration on Hela Cell Viability After Incubation 72 hours

Concentration	Viability	Elementary school
1. 5625	114,9654	0.0639
3. 125	102.3924	0.0093
6. 25	90.9399	0.0044
12. 5	45,337	0.0026
25	12.44747	0.0038
50	12.1725	0.0060
100	11.6029	0.0020

Discussion

Effect of leaf extract nggorang on the viability of HeLa cells is determined by the number of hela cells were still alive after nggorang leaf extract incubated together with various variations. *HeLa* cells that are still alive will look like elongated and clustered leaves attached to the bottom of the well. Meanwhile, dead *HeLa* cells will form irregular dots and float on the media. After treatment with the MTT method, will be seen formazan crystals that show *HeLa* cells that are still alive[5].

Giving extracts ranging from a concentration of 31,675 ppm to 1000 ppm. More and more living cells show that the concentration of extracts of leaves given by people is not effective. Likewise the concentration of extracts that provide viability of Hela cells is very little is an effective dose. This is consistent with the statement that cell viability is the number of cells capable of developing in a culture medium. Cell viability is used as a marker of cytotoxicity of a material to determine the biological properties of a material that is toxic to certain cells. One that indicates the cytotoxicity of a substance is a decrease in cell proliferation and decreased viability[6].

The longer incubation, the more T47D cells die, so the EDG dose needed to kill T47D cells gets smaller because the number of living cells has decreased. This means that the use of EDG drugs has the potential to be anti-cancer. In this study the IC50 value of EDN on Hela cell viability was obtained at 187.00 ppm. This means that a dose of 187.00 ppm was quite effective in killing Hela cells.

The activity of EDN in killing cancer cells is thought to be related to the content of active substances including flavonoids. The mechanism of flavonoids in inhibiting tumor/cancer proliferation is to inhibit the activity of tyrosine kinase receptors so that they can inhibit the signal transduction pathway from the membrane to the cell nucleus. Increased tyrosine kinase receptor activity plays a role in the growth of cancer cell malignancy. Besides flavonoids also function to reduce tumor resistance to chemotherapy agents[7].

As a comparison in this study, the anti-cancer drug doxorubin was used. The effect of doxorubin on cell viability showed analogous results with the results of EDG on cells. IC50 value of doxorubin administration on viability of hela cells after 72 hours incubation was 149.00 ppm. While the IC50 value of EDG on cell viability after 72 hours incubation was 149.00 ppm. Based on this, it can be stated that EDG can potentially be as anti-cancer as doxorubin.

The results of observations of the effect of EDG administration on P53 gene expression in helper cells showed that cells expressing brownish P53 genes at their core. This result is different from cells that do not express the P53 gene. The data shows that the higher the EDG concentration given, the greater the hela cells expressing the P53 gene. So it can be stated that EDG has the potential to increase the expression of the P53 gene in cancerous cells. P53 gene expression in cells that appear brown is a P53 gene with wild type in cytoplasm that plays a role in the process of cell apoptosis[8].

In the study it was found that EDG has the potential to cause apoptosis in cancer cells in concentrations of 10ppm - 50 ppm. EDP 50ppm potential as a haem cell apoptosis because the number of cells expressing the P53 gene is 92.05%. In the event of apoptosis the P53 gene is expressed when DNA damage occurs so that an error occurs in the cell cycle that is the P53 gene induces cell

cycle arrest. It is intended that DNA damage in damaged cells has time to be repaired. But if the damage cannot be repaired then the expression of the P53 gene will increase so that it will trigger cell apoptosis (Hanahan and Weinberg, 2000). Conversely, if the P53 gene is not expressed in cells, then a mutation occurs in p53 so that the gene becomes unstable and has the potential to become cancerous. The p53 gene is a signal of apoptosis because it suppresses the formation of tumor formation and inhibits malignant progression. The mechanism that occurs in cells is the p53 gene eliminates cells that have DNA damage that has the potential to become cancerous cells by cell cycle arrest, apoptosis or repair[9].

Conclusion

Nggorang Leaf Extract (EDG) has potential as an antioxidant against DPPH radicals at IC50 = 34,407 ppm

1. Nggorang Leaf Extract (EDG) has the potential as an antiproliferation of Hela IC50 cancer cells = 187 ppm
2. Nggorang Leaf Extract (EDG) has the potential to increase P53 gene expression in Hela cancer cells by 92.05% at a concentration of 50 ppm.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: Nil

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