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Cytotoxicity and Antioxidative Effects of Ethanolic Extract Green and Red Cultivar Ganyong Rhizome (*Canna indica* Linn) against Colon Cancer Cell Line *WiDr*

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***Abstract*-Ganyong is a food crops grown in Indonesia. Ganyong rhizome often called ganyong tuber has been used as food by people in antiquity. Now it's the utilization of tuber of this plant as food less interest. Ganyong tuber has a high fibre content, butyric acid and there is some secondary metabolite compounds include: alkaloids, flavonoids, saponins, steroid and triterpenoid, phenolic. The Group of compounds most have activity as antioxidant and anticancer. Colon cancer sufferers in the world occupy third place, and will expected to go up in number due to dietary changes and less unhealthy activity. Ganyong who grew up in indonesia is composed of two varieties red and green ganyong and differences between these two varieties have not been much studied.**

Keywords-antioxidative, canna indica, colon cancer, cytotoxicity, WiDrcells

I. INTRODUCTION

Canna indica Linn (Ganyong), is a food crop that its existence is scant attention. In antiquity, many communities that cultivate these plants in around the house for exploited bulbs or leaves, but will now uncommon and rare. This plant shaped herbs and rhizoma has often referred to as tuber ganyong. Ganyong bulbs can be consumed with boiled proceses and can be also made of starch [20]. Lately, the expediency of tuber starch or ganyong many examined primarily in the fields of industry as well as in the field of health.

Ganyong plant in java and Central Moullucas have 2 cultivare green and red[20]. Both of these cultivars are also found in Bengkulu[18] and other regions such as NTB, Kalimantan and Sumatra[9]. A fundamental difference in the two cultivars was found on the rhizome. Red Ganyong has based rhizome is red. Green ganyong color is white rhizome. Difference content of tuber ganyong red and green is located on a number of moisture content, ash, protein, fat, carbohydrates and total fiber. Moisture content, ash, protein and fat in the tuber ganyong red ganyong bulbs greater than green[2].

Nutritional tuber ganyong contained 95 calories, 1 g protein, 0.1 g fat, 226 g carbohydrates, 21 mg calcium, phosphorus-70 mg, iron 20 g, 0.1 mg of vitamin B1 and vitamin C 10 mg per 100 g of ingredients[13]. In addition, the ganyong bulbs have high fiber content as much as 10.4 g[15] Other macro-nutrient compounds contained in the tuber ganyong is butyric acid[7]. Phytochemical contents in ethanol extract of tuber ganyong: alkaloids, flavonoids, saponins, steroids, terpenoids, fixed oils, phytosterols, phenolic, fats, carbohydrates, proteins, glycosides, Tannins[5] phlobatinin, betulinic acid, oleonolic acid, lignin, furfural and hemicellulose [6];[1].

Compounds are secondary metabolites that are known to have anti-cancer activity as follows: alkaloids, flavanoid, diterpenoid, and polyphenols[10]. Based on the diversity of compounds contained in ganyong, the possible types of this plant can be used as ingredients of prevention against cancer occurrence. In addition to anticancer, anti-viral activity is also found mikrobia from the oil of life of rhizoma ganyong[4].

The activity of this extract acts as free radical oxidative. Other activities that are found from the extract of tuber heksan ganyong sitotoksisitas activity against P388 leukemia cancer cells[3]. In addition to the activity of the compounds of secondary metabolites from ganyong tuber, known activity of starch gayong in inhibition of the growth of colon polyp dysplasia[18].

The number of cases of cancer of the colon and rectum (colorectal) ranks third in the world by the year 2012. Colorectal cancer mortality in the U.S. based on the American Cancer Society estimated at 49,700 people in 2015[13]. The cause of this cancer is triggered by genetic and environmental factors. Environmental factors trigger from events of this cancer is a lifestyle and diet food. According to data from the Globocan, colon cancer Incidence is estimated to be rising due to dietary changes and unhealthy lifestyle are less physical activity[16].

The activity of starch gayong is already known as a preventive agent against colon cancer incidence in vivo, but the activity of compounds of secondary metabolites found in the tuber is not yet known. Therefore, it is necessary a study

that examines the roles of secondary metabolites found in the tuber ganyong especially as anticancer. The purpose of knowing the particular activity of cytotoxixity between the two varieties of coarse ganyong extract with chloroform and ethanol solvents compared faction – the fraction obtained from extracts of rough most potential as anticancer colonic.

II. METHOD

A. Plant Material

Green and Red ganyong rhizome collected during dry season in 2017 from Boyolali, Central Java, Indonesia. Rhizome removed from soil and sliced. Material dry out with oven at 50 ^{0}C for 5 days. Drying material grounded into powder

B. Chemical and Reagents

Ethanol, Methanol (PA Merck), DMSO, RPMI 1640 (Sigma- Aldrich), Fetal Bovine Serume , Phosfat Buffer saline (PBS), 0.25% Trypsin mg/ml trypsin-EDTA, MTT Reagent(3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazoliumbromide) 50 mg in 10 ml of PBS, the SDS in 0.01 N HCL, DPPH and Doxorubicin.

C. Tools

Ovens, clear plastic, dark colored glass bottles, filters, vacuum with rotary evaporation, analytical , hood, micro tube, petri dish, mikropipet, CO2 incubators, microscopes, LAF, autoclave, elisa reader and Uv-vis spectrophotometer.

D. Procedur

1. Extraction of Ganyong Rhizome

Rhizome powder made from the fresh rhizome dryer during 4-5 days. Rhizome sliced then beaten afterwards sifted on the size of 40 mesh. Ganyong rhizoma powder with weight 200 g macerated with ethanol solvent in dark bottled as long as 72 hours and shaken out once in a while every day. Extract is filtered using the filter paper while vaccum. Extract evaporated by the rotary evaporator at a temperature of 64^{0}C. The extracts obtained were weighed and put in a glass container and stored in the refrigerator. The extract obtained the extract of rhizome green and red cultivars with ethanol solvent.

2. WiDr cell Preparation

*WiD*r cells prepared from stock cultures are stored in tanks of liquid nitrogen. Then the cells grown on some of the flask or a petri dish tissue culture and incubated at 37 ^{0} c and keep in CO_2 incubator after 24 hours the medium grown cells to replaced and konfluen 70-80% and the amount is enough to research. After the amount of enough to konfluensi every plate reaches 70%, the cell replaced from medium and washed with PBS and added approximately 1 ml trypsin while leveled. Petridish incubated about 5 minutes. After it added as much as 5 ml RPMI medium and dihomogenkan. Cell cell harvesting results calculated by count haematocytometer booth. Once known the number of estimated total cells, then prepared for the purposes of planting on the plate as much as 1x10^4/100 µl to 96 well plate with[16].

3. Cytotoxixicy Test of crude extract

Cells that had been grown on a 96 well plate for 24 hours view their condition. Ganyong rhizome extract 10 mg first added as many as 100 µl DMSO as a stock solution with a concentration of stock 100,000 ppm. From new stock solution diluted with RPMI medium at concentrations (ppm) of 2000;1500;1000; 750; 500; 250; 125. Cells in plate disposed mediumnya, after it is added to each well with 100 µl mix medium with different concentrations of extracts. Then incubated for 24 hours. After it was seen under a microscope how influence on cells. Calculation of living cells with MTT assay test. After being seen in a microscope, the cells are then disposed of mediumnya after it added MTT reagent that has been dissolved in 1 ml of PBS plus 9 ml RPMI medium, as many as 100 µl/well. Once it was incubated for 4 hours. After it added 100 µl/stopper reagent well 10% SDS in HCN 0.1 n. After it wrapped with paper and left overnight. Absorbance readings done by elisa reader with 595 nm λ (UGM medical parasitology Lab.) cell Viability can be known by the method MTT. Living cells can form blue formazan activity reduction of 3-[4,5-dimethylthiazol-2-yl]-diphenyl tetrazolium bromide-2.5 (MTT) by the enzyme dehidrogenasi on mitochondrion[8].

4. Test antioxidant activity with the DPPH assay

Stock solution made in concentration100 ppm with dissolving 10 mg extract in 100 ml methanol PA. Further dilution using solvent methanol concentration variation. Prepare a solution of DPPH stock 50 ppm. Stock solution of DPPH is made by dissolving 5 mg solids DPPH into 100 ml methanol PA. Then the prepared solutions of the comparison, i.e. the control solution containing 2 ml of methanol PA and DPPH solution 1 ml of 50 ppm. Standart solution used L- Ascrobic Acid. For the test sample, prepared each of 2 ml of the sample solution and 2 ml solution of DPPH. Later, incubated for 30 min at a temperature of 27 ℃ until the color changes from DPPH activity. All sample i.e. the sample extract that has been in incubation at its absorbance value test using Uv-vis spectrophotometer at a wavelength of 517 nm

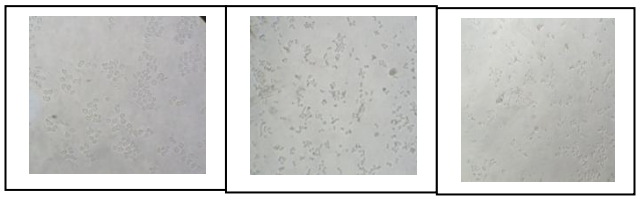
E. Data Analysis

Cell viability data analyzed by probit analysis to get the value of the Ic $_{50.}$ While the value of the validity of the measurement results seen with the curve inclination with a value of R is approaching 1. The selected extract is a kind of extract of rhizoma of cultivars of a particular type of solvent that has the lowest value of Ic_{50} against WiDr cells.

III. RESULTS AND DISCUSSION

A. Result

The results obtained from this research is the toxicity of the extract ethanolic of rhizome ganyong green and red cultivare against colon cancer cells *WiDr*. Figure 1. Differentiation morphological WiDr cell control (A) and added extract ganyong two cultivare green (B) and red (C).



A B C

Figure 1. Differentiation cell control *WiDr* cell (A) with cell added green ganyong extract (B) and red ganyong extract (C).

Treatment of ethanol extract ganyong either red or green giving effect on the cells that are different in terms of size, shape and number of living cells. Ethanol extracts of green ganyong inhibits the growth of some WiDr cancer cells and deadly greater than ethanol extract ganyong red. The amount of cell death detected by the method MTT.

Living cells will be detected with the formation of crystals of formazan. Dead cells wasn't forming formasan crystal. Calculating living cells showed by absorbance from crystal formazan. The concentration of extract causing cell death of 50% will be calculated as the value of Ic_{50}. Figure 2, shows the great value of the ethanol extracts of Ic_{50} ganyong red and green. kill 50% of the cells are generally called Ic_{50}.

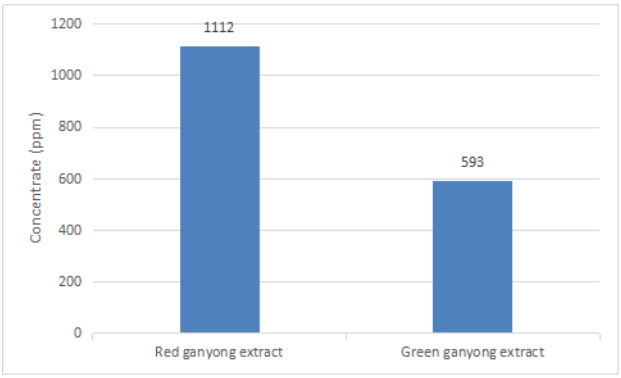


Figure 2. Ic_{50} value red ganyong ethanolic extract and green ganyong against WiDr cancer cell line.

Ic_{50} values extract green ganyong 829,25 ppm and 1031,75 ppm to extract ganyong red. Ic_{50} values green ganyong extract Ic50 value is smaller than the red ganyong. These results indicated the green ganyong more toxic extract against WiDr cancer cells than red ganyong extracts. The nature of the toxicity that is owned by green ganyong extract gives information that is potentially as a colonic anticancer.

The anticancer potential of one due to the antioxidant content. Therefore, tested antioksidanya activity as one indicator of potential anticancer. Antioksidan activity test is performed with the DPPH test. Test results can be seen in Figure 3.

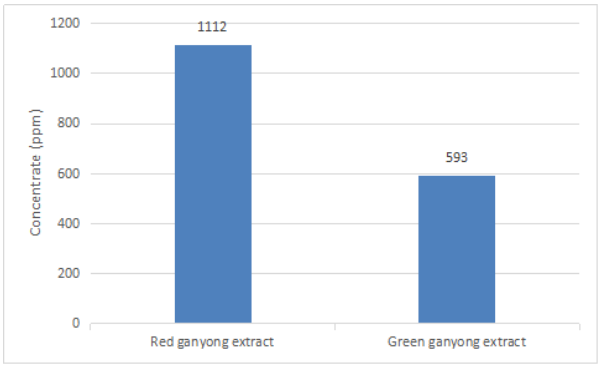


Figure 3. Ic_{50} value red ganyong ethanolic extract and geen ganyong absorbed free radical from DPPH.

The result in Fig.3 showed Ic_{50}values of absorption DPPH of green ganyong ethanolic extract at 593 ppm and 1.112 ppm to red ganyong extract. Ic_{50} values extract green ganyong smaller than the value in the ganyong extract of red. Smaller values indicate greater antioxidant activity than red ganyong extract.

B. Discussion

The research results showed that due to the addition of ethanol extract of ganyong on cell line *WiD*r showed a disruption. Effect of addition of extract of etanolic ganyong good green or red cell shape change and causes, measuring a bit more refined. The shape of cells disrupted by instability of causing components in colon cancer cells. Instability in the cells could cause cancer cell death. The presense of the cancer cell death due to treatment of specific compounds used as models in the search for compounds that have anticancer activity.

WiDr cell line can experience the death of 50% can be achieved at a concentration of treatment to 829.25 ppm of ethanol extract of rhizome green ganyong and 1031.75 ppm for ganyong red. This toxicity value belongs to high but still in the range of under 2000 ppm. The range of under 2000 ppm still meets the criteria as a cancer preventive foodstuffs.

Cell death can be known from his cell component activity namely mitochondria. The mitochondria organelle is the basic menadakan of life cells. The activity of the cell metabolism in the mitochondria can show the life of cells. Cells alive doing the active enzyme and metabolism of NAD (P) H-dependent cellular oxidoreductase. This enzyme that can berekasi with MTT reagent forming crystal formazan [12]. Living cells are tested with MTT will produce purple Crystal from formasan. Dead cells do not react with MTT. It is this distinction that is used as an indicator of the cell life on this test.

Compounds that potentially as most have anticancer activity as antioxidant. The value of antioxidant activities of ethanol extracts of green ganyong greater than ganyong red extracts. Ic_{50} values 593 ppm range including the very weak [21]. Secondary metabolite compounds that exist in ethanol extracts, among others; alkaloids, flavonoids, saponins, steroids, terpenoids, fixed oils, phytosterols and phenolics. Among this group of compounds, compounds that have anticancer activity among other alkaloids, these Terpenoids and phenolic resins [11].

Some compound have antioxidant activity, can destroy cancer cell DNA [22]. Destroying DNA induced cell death. Apoptotic and necrotic is cells death pathway. The effect of herbs treatment caused cell death.

The screening anticancer compounds with the phytochemical material, generally used this method. The ideally cell death pathway in this model is apoptotic pathway. Apoptotic cell death didn't caused inflammation. This treatment model is safer for people with cancer or also as preventive measures against the occurrence of cancer.

IV. CONCLUSION

Ethanol extract of rhizome ganyong are toxic against WiDr colon cancer cells. Rhizome extract green ganyong more toxic than rhizome extract red in addition antioxidant aktivity also higher than red ganyong. Rhizome ganyong potential as anticancer colonic foodstuff.

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REFERENCES

[1] Al-Snafi. A E, Bioactive components and Pharmacological effects of *Canna indica*-an Overview. *ijpt*. Vol :5(2) pp 71-75, 2015.
[2] Carolina, A dan Ilmi, F.N. Production of Indonesian Canna edulis type IV resistant starch through acetylation modifation. IFJR Vol: 23 (2) pp 491-497, 2016.
[3] Chainakul S, Soukharath V, Tambunchong C, and Picha P. Study on cytotoxicity of the hexane crude extracted from the rhizome of Canna indica Linn. on cancer cells [2001] http://agris.fao.org/agris-search/search.do?recordID=TH2005000496,2001.
[4] George J. Screening and antimicrobial activity of Canna Indica against Clinical pathogens bioactive. IJLSER Vol:2(3) pp.85-88, 2014.
[5] Jeyaraman. V, Muthukkumarasamy.S and Antony Joseph Velanganni. A. Phytochemical analysis of Canna indica L. IJONS. Vol. 1(5), 2011.
[6] Lamaeswari G and Ananthi T. Preliminary phytochemical screening and physiochemical characterization of Canna *indica* L. *Int J Pharm Sci Rev Res*. Vol:14(2) pp. 76-79, 2012.
[7] Lim TK. Edible medicinal and non-medicinal plants modified stem , roots Vol: 10, 2016.
[8] Sari N, Purnomo, Daryono B.S, Suryadiantina, and Setyowati M. Variation and intraspecies clasification of edible canna(Canna indica L) based on morphologycal characters. AIP Conf.Proc.1744.020041-1-020041-8, 2016.
[9] Solowey E, Lichtenstein M, Sallon S, Paavilainen H, Solowey E, and Lorberboum-Galski H. Research Article: Evaluating medicinal plants for anticancer activity. The Scientific Word Journal.Vol:2014: Hindawi Publishing Corporation, 2014.
[10] Anonim. Badan ketahanan pangan dan penyuluhan provinsi DIY, 2014.
[11] Anonim. Direktorat Gizi Depkes RI. Daftar komposisi bahan makanan. Bharata : Jakarta, 1989.
[12] Anonim.GLOBOCAN.http;//globocan.iarc.fr/default.aspx, 2012.
[13] CCRC.http://ccrc.farmasi.ugm.ac.id/wp-content/uploads/03.010.02-uji-sitotoksik-MTT.pdf, 2009.
[14] Ismawardika A, Larasandi D, Sadid A, Fiolita R E, Odiyana N and Griadhi P. Ganyong (Canna edulis), yang terabaikan menjadi benteng kanker usus besar. Prosiding Elektronik Program Kreativitas Mahasiswa. Artikel.dikti.go.id>PKM-P>article>view, 2013.
[15] Suhartini T dan Hadiatmi. Keragaman karakter morfologi tanamam Ganyong. Buletin Plasma Nutfah Vol:16 (2). 2010.