DOI: http://dx.doi.org/10.33846/hn50504
http://heanoti.com/index.php/hn

RESEARCH ARTICLE
URL of this article: http://heanoti.com/index.php/hn/article/view/hn50504

Gondomono (*Hedycium spicatum*) Ethanol Exstract Anti-Proliferation, Apoptosis and P53 Cancer Cell Line

Nur Rahman
Center of Excellence for Science and Technology on Local Materials for Handling Non-Communicable Diseases, Poltekkes Kemenkes Malang, Indonesia; rahmancahaya@yahoo.com (Corresponding Author)

ABSTRACT

The incidence of cancer from year to year continues to increase. Some of the causes of this incident include an unhealthy lifestyle, smoking, a diet high in fat and low in fiber. The Gondomono plant (*Hedychium spicatum*) are the most studied species. Interesting compounds have been identified like coronarin D, which possesses antibacterial, antifungal and antitumor activities, as well as isocoronarin D, linalool and villosin that exhibit better cytotoxicity towards tumor cell lines than the reference compounds used, with villosin not affecting the non-tumor cell line. This study aims to obtain scientific evidence that Gondomono extract (*Hedychium spicatum*) is proven to be anti-cancer. The impact obtained will be utilized by local Indonesian plants that can be processed or produced as functional food and herbal medicine for anti-cancer bioactivity toxicity with various tests including the making gondomono (*Hedycium spicatum*) flour, ethanol extraction method, insilico analysis and Gas Chromatography-Mass Spectrometry (GC-MS) analysis, then the second stage test, namely the MTT test, P53 test and apoptosis test. The research design was experimental. Gondomono extract when compared to anticancer ingredients was indeed very far away, gondomono extract was starting to be able to act as apoptosis, it requires a fairly high concentration, from 200 to 400 ppm. As conclusion, the higher the dose of gondomono extract, the higher the expression of p53 protein, the higher. Gondomono extract as a cancer cell killer requires a minimum dose of 368.51 ppm.

Keywords: gondomono; *Hedycium spicatum*; anti-proliferasi; cancer cell line

INTRODUCTION

Background

The incidence of cancer from year to year continues to increase, in 1996 there were 10 million new cases, and of these cases 6 million died, it is estimated that in 2020 new cancer cases will be 20 million and 12 million died. Some of the causes of this incident include an unhealthy lifestyle, smoking, a diet high in fat and low in fiber [(1)]. Breast cancer is a cancer that is the leading cause of death, with 23% of cases and 14% of deaths, while the second place is lung cancer, with 17% of lung cancer cases and 23% of deaths [(2)].

In the United States as a developed country, cancer is the second leading cause of death (23.1%) after heart disease (26%). In the group of women, breast cancer is the second cause of death (15%) after lung cancer (26%). In the male group, lung cancer is the first cause of death (30%). There is a tendency that from year to year the death rate due to cancer continues to increase, starting in 1930 by 50,000 people and in 2006 to 280,000 people [(3)].

In 2010, the prevalence of tumor/cancer in Indonesia was 1.4%. Among non-communicable diseases, cancer is in the 4th rank, equivalent to diabetes mellitus with a prevalence of 10.2% (n = 2,285) after stroke, hypertension, and ischemic heart disease. Cancer is the 6th cause of death, equivalent to diabetes mellitus by 5.7% after stroke, tuberculosis, hypertension, injury and perinatal disease [(4)]. The number of cancer patients in Indonesia is estimated to continue to increase from year to year with an estimated number of 12 million people in 2030.WHO also said that every year there are around 6.25 million people with new cancer and this number will continue to increase if there is no treatment action. and prevention of cancer [(5)].

The results showed that consuming a diet high in fruit and vegetables will fight cancer. From the results of 200 studies stated that vegetables and fruit were significantly found 128-156 studies associated with a reduced risk of cancer. The results also show that consumption of vegetables and fruit can reduce approximately 20-23

percent of lung cancer [6]. Therefore, based on the description above, a research on the potential of plant mixture extracts as anti-proliferation of cancer cells will be carried out.

Purpose

The long-term objective of this study was to obtain a method of extracting plant mixtures, as well as obtaining scientific evidence that mixed plant extracts can be used as an anti-cancer prevention.

METHODS

Design

This study was an laboratory research. The research was conducted from June to December 2016 in the laboratory of UIN Malang, Brawijaya University biomedical laboratory, UM chemistry laboratory, and Polinema chemistry laboratory. The material used was a healthy, green, gondomono plant (*Hedycium spicatum*) taken from the Batu area, East Java.

Testing Phase 1

Making Gondomono (*Hedycium spicatum*) Flour

Weighing 1 kg of gondomono (*Hedycium spicatum*) plants, oven at 55° C for 1 day. After drying, the gondomono plants were ground in a blender and sieved. Furthermore, the gondomono flour would be extracted with ethanol [7].

Ethanol Extraction Method

Weighing 100 g of gondomono flour that had been smooth. Then extract (macerated) with ethanol 70% 7 x dry matter (50 g), with stirring for 2 hours and left to stand for 1 night. The solution was then filtered with Whatman filter paper No. 1, the solvent was removed or evaporated by an evaporator at a temperature of 50°C [8].

Insiloco Analysis

Analysis of biological activities based on the value of Probability activity (Pa) with a value range of 0 to 1. Using tools: PyMol and PyRx [9].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Determination of the structure of the compounds of gondomono (*Hedycium spicatum*) was carried out using a GS-MS spectrometer. The analysis was performed using GC-MS Agilent 6.890 N 2006 with 5 MS DB column dimensions of 30 m x 250 µm x 0.25 µm. The system conditions were oven temperature 310°C, detector 310°C, injector 305°C, program temperature (initial 50°C, increase of 3.50°C per minute to a maximum temperature of 310°C), analysis time 100 minutes, column pressure 14.12 psi with a flow rate of 1.7 mL per minute and an injection volume of 2 µL[10].

Testing Phase 2

MTT Test Method

This study aimed to examine the inhibition of gondomono extract against the proliferation of servic cancer cells, using the MTT (3- (4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide) method. The MTT method was used to determine which cells are still alive. In measuring the color change by measuring the activity of an enzyme that reduced MTT to formazan which gives a purple color MTT yellow dye reduced the purple color of formazan in living cells [11]. Cancer cells in complete medium (DMEM) were added with fetal bovine serum 10%. Penicillin 100 µ / ml and streptomycin 100 µg / ml) were cultured for 24 hours. After the cell density reached about 50%, the cultures were further incubated for 48 hours with or without extract treatment at concentrations of 25, 50, 100, 200, 400, 800 ppm medium with IC50. Testing the inhibitory activity of cancer cells was measured by an ELISA reader at a wavelength of 570 nm [12]. The positive control used docsorubin with concentrations of 0.005, 0.1, 0.5 1, 5, 10, 25, 50, 100 nM[13].

P53 Test

The p53 protein is a tumor suppressor protein that acts as a regulator of the cell cycle. The p53 protein plays an important role in the response to cellular stress, such as exposure to carcinogens. This protein will inhibit the proliferation of abnormal cells that have been initiated by carcinogens to prevent the development of neoplasms. The inactivity of these proteins can cause malignancy to malignant cancer. Besides functioning to

regulate cell proliferation, p53 also regulates apoptosis, inhibits angiogenesis, and regulates DNA repairment. In cancer, generally p53 has a mutation. The most common p53 mutation is missense mutation. These mutations can be in the form of p53 degradation, loss of p53's ability to induce cell cycle arrest or apoptosis, and loss of p53's affinity for binding to damaged DNA. Immunohistochemistry is a process of identifying specific proteins in tissues or cells using antibodies. The site of binding between antibodies and specific proteins is identified by markers that are usually attached to the antibody and can be visualized directly or by reaction to identify markers. Markers can be colored compounds, fluorescent substances, heavy metals, radioactive labels, or enzymes [(14)].

Apoptosis Test

A number of cover slips were prepared and then inserted into the 24 wells microplate. The number of cell suspensions (5 x 105 cells) in complete culture media was transferred as much as 1 ml onto the cover slip. The distributed cells were put in a CO_2 incubator at 37°C overnight. After one night, the media containing the cells was discarded and the test sample was inserted into the well as much as 1 ml. The microplates were then incubated for 10 hours in a CO_2 incubator. The microplates that have been incubated are removed from the incubator and then the media in the well is discarded. The cells in the well were washed with 1 ml of PBS. PBS from the well removed with a micropipette slowly. The cover slip was carefully removed and then placed on the glass object. A total of 10 µl of reagent mixture of ethidium bromide - acridine orange was dropped on the cover slip. The cells were then observed under a fluorocene microscope (Olympus) [(15)].

RESULTS

Qualitative Test

Table 1 shows that the Gondomono extract qualitatively contained flavomoid, alkaloid and saponin ingredients.

Table 1. Gondomono extract qualitative test results

No	Parameter	Result
1	Flavonoid compounds	Positive
2	Alkaloid compounds	Negative
3	Saponin compounds	Negative

Antioxidant Activity Quantitative Test

Table 2. Antioxidant activity of gondomono extract

Concentration (ppm)	% Inhibition (Repeat)			Average
	I	II	III	
24	7.26	8.33	8.38	7.99
48	17.32	17.78	17.88	17.65
72	26.82	28.33	26.26	27.13
96	32.96	33.89	32.96	33.27
120	38.55	40.00	39.11	39.21
IC50 (ppm)	149.99	145.51	150.65	148.72

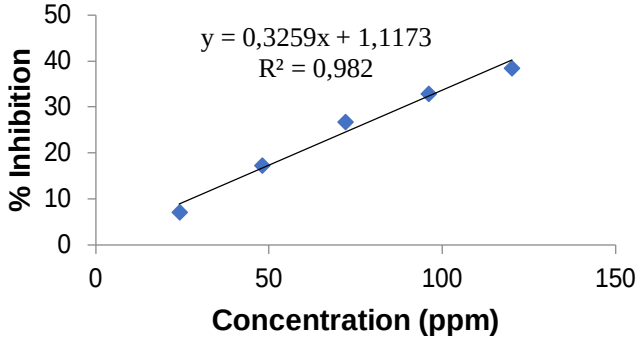


Figure 1. Antioxidant activity of gondomono extract

In table 2 it can be seen that the Gondomono extract had an IC50 of 148.72

Bioactive Component Test

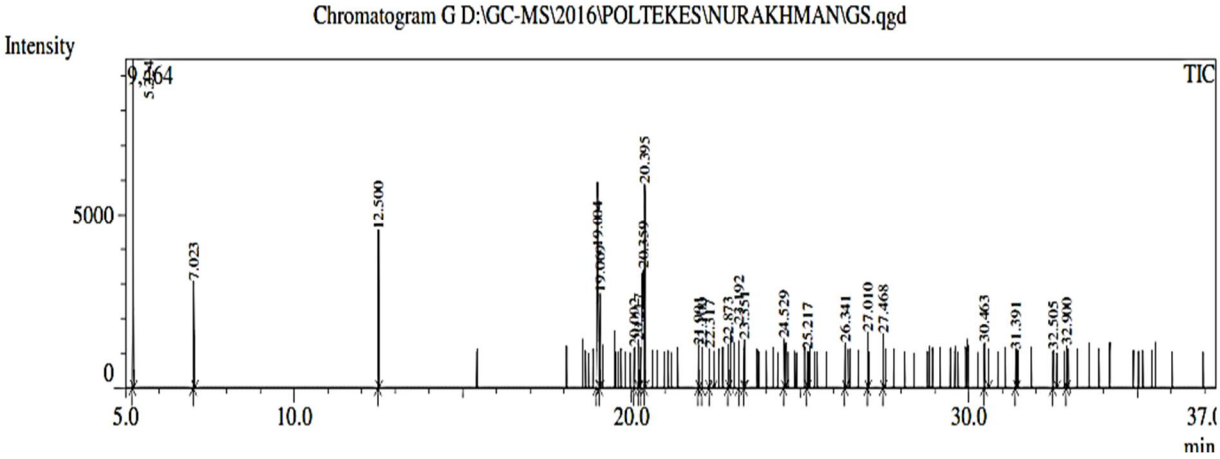


Figure 2. Bioactive component test activities

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H
1	5.224	14659	15.44	9464	17.27	1.54
2	7.023	4805	5.06	3063	5.59	1.56
3	12.500	5264	5.54	4558	8.32	1.15
4	19.004	13779	14.51	4024	7.34	3.42
5	19.069	3266	3.44	2720	4.96	1.20
6	20.092	1152	1.21	1185	2.16	0.97
7	20.217	1814	1.91	1331	2.43	1.36
8	20.359	11958	12.60	3406	6.21	3.51
9	20.395	10952	11.54	5887	10.74	1.86
10	21.991	1647	1.73	1220	2.23	1.35
11	22.100	1166	1.23	1183	2.16	0.98
12	22.317	1099	1.16	1128	2.06	0.97
13	22.873	1726	1.82	1253	2.29	1.37
14	23.192	1219	1.28	1359	2.48	0.89
15	23.351	1863	1.96	1397	2.55	1.33
16	24.529	2544	2.68	1404	2.56	1.81
17	25.217	1560	1.64	1065	1.94	1.46
18	26.341	1813	1.91	1292	2.36	1.40
19	27.010	1993	2.10	1612	2.94	1.23
20	27.468	1924	2.03	1564	2.85	1.23
21	30.463	2378	2.50	1291	2.36	1.84
22	31.391	2660	2.80	1098	2.00	2.42
23	32.505	2059	2.17	1093	1.99	1.88
24	32.900	1635	1.72	1207	2.20	1.35
		94935	100.00	54804	100.00	

Figure 3. Bioactive component test activities

[Comment]

===== Analytical Line 1 =====

[GC-2010]

Column Oven Temp.	:100.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Pressure
Pressure	:100.0 kPa
Total Flow	:537.7 mL/min
Column Flow	:1.33 mL/min
Linear Velocity	:43.0 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:400.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	100.0	2.00
15.00	280.0	2.00
15.00	300.0	20.00

< Ready Check Heat Unit >

Column Oven	: Yes
SPL.1	: Yes
MS	: Yes

< Ready Check Detector(FTD) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >

SPL.1 Carrier	: Yes
SPL.1 Purge	: Yes

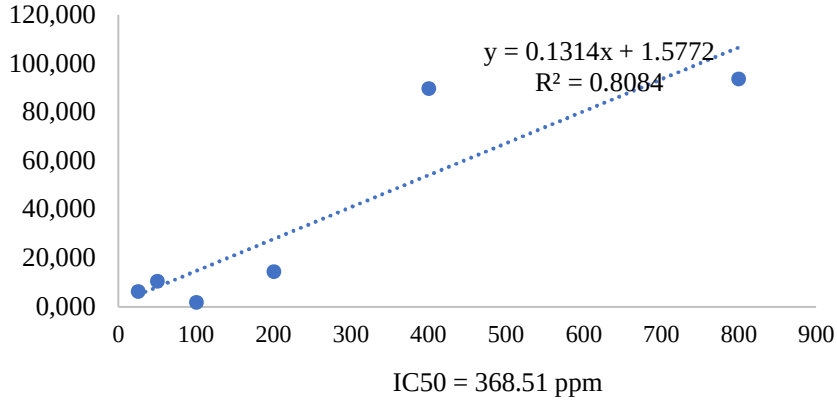
< Ready Check APC Flow >
< Ready Check Detector APC Flow >

Figure 4. Bioactive component test activities

MTT Proliferation Test

Table 3. MTT method proliferation test results

No	Concentration (ppm)	Percent cell death
1	25	6.27
2	50	10.380
3	100	1.81
4	200	14.506
5	400	89.88
6	800	93.63
7	Cisplastin (17.1 ug/ml	86.82



IC50 = 368.51 ppm

Figure 5. MTT method proliferation test

In Figure 5, it can be seen that the IC50 value of the Gondomono extract was 368.51 ppm, this value was sufficient (note: good if the IC50 value is below 300 ppm).

Apoptosis Test With FITC Anexin Method

Table 4. Apoptosis test with FITC anexin method result

	Concentration (PPM)				Avarage
	I	II	III	IV	
0	1.56	1.95	2.63	1.95	
25	2.03	1.88	1.46	1.71	
50	1.81	1.85	1.93	1.87	
100	2.78	2.90	2.82	3.27	
200	13.59	7.35	7.09	10.30	
400	78.79	86.90	86.68	86.50	
800	90.05	89.99	90.00	89.64	
K+	70.97	71.01	67.02	66.32	

In Table 4, it can be seen that giving a concentration between 200 to 400 could kill cancer cells which was equivalent to a positive control, namely the could kill cancer cells by 84.7%.

P53 Test

Table 5. P53 Test Result

	Concentration (PPM)				Avarage
	I	II	III	IV	
0	64.41	62.10	62.17	59.21	
25	64.41	62.10	73.27	77.18	
50	75.72	75.60	81.26	81.87	
100	95.49	94.73	95.26	96.11	
200	88.94	90.41	93.56	91.70	
400	90.12	90.41	87.29	89.02	
800	98.81	98.80	98.68	99.15	
K+	32.77	26.90	49.81	55.45	

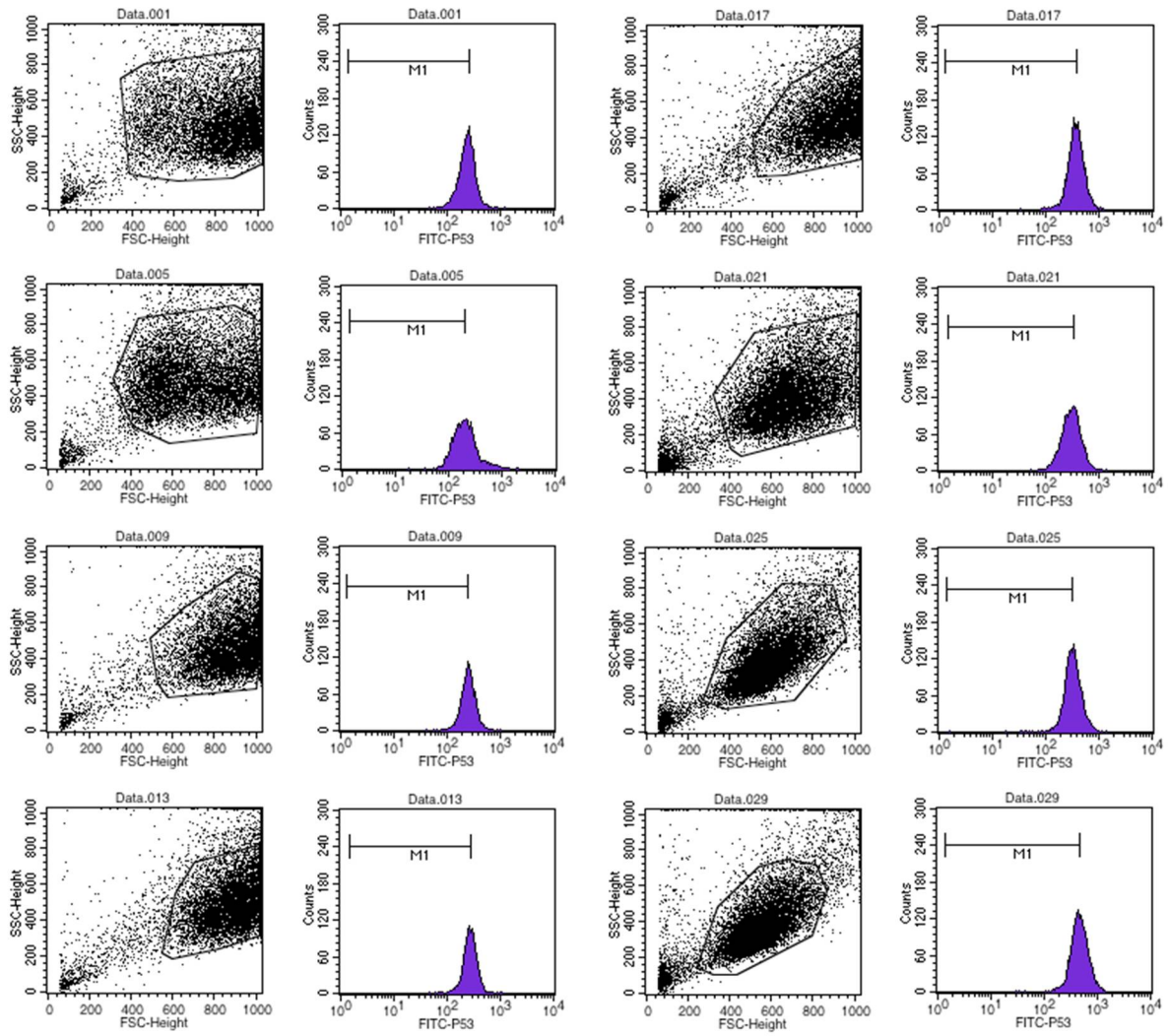
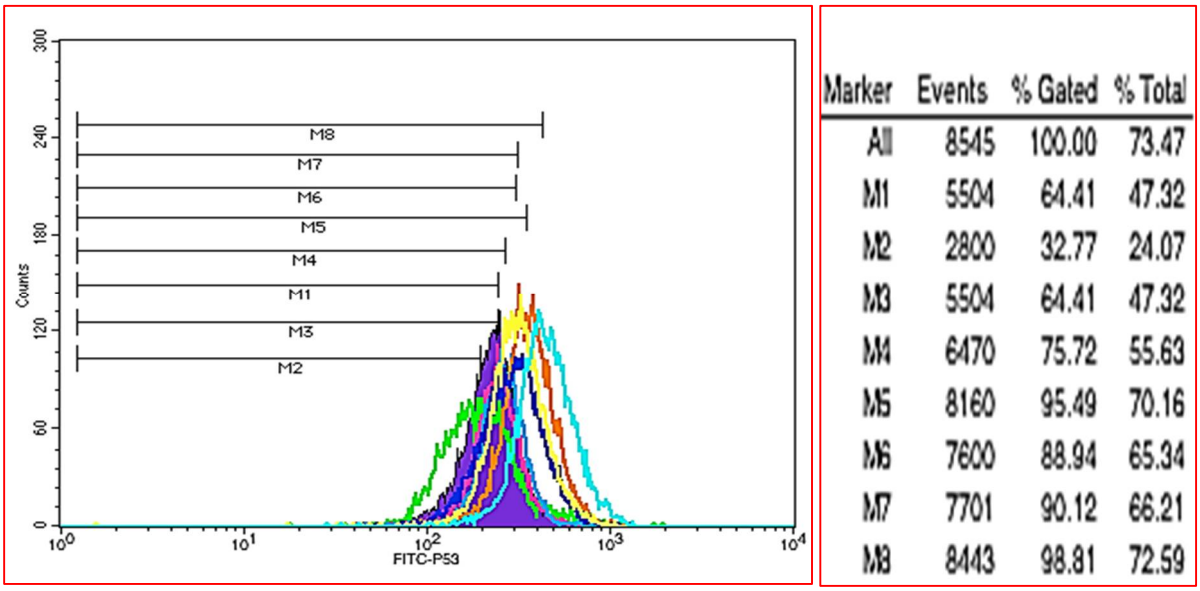


Figure 6. P53 test activity



Marker	Events	% Gated	% Total
All	8545	100.00	73.47
M1	5504	64.41	47.32
M2	2800	32.77	24.07
M3	5504	64.41	47.32
M4	6470	75.72	55.63
M5	8160	95.49	70.16
M6	7600	88.94	65.34
M7	7701	90.12	66.21
M8	8443	98.81	72.59

Figure 7. P53 Test Activity

DISCUSSION

The results of the qualitative analysis showed that the gondomono extract contained flavonoids, alkaloids and saponin components. Gondomono extract has an IC50 of 148.72 ppm. Gondomono extract has an IC50 of 148.72 ppm. IC 50 on cancer cells of Gondomono extract was 368.51 ppm. The effect of giving gondomono extract will affect the apoptosis value, with positive control with a dose of 1.7 ppm, the apoptosis value is 68%, with the provision of gondomono extract the apoptosis value is around 84.8 at a dose of 400 ppm, when compared with the standard apoptosis value is very far.

Gondomono extract when compared to anticancer ingredients is indeed very far away, gondomono extract is starting to be able to act as apoptosis, it requires a fairly high concentration, from 200 to 400 ppm. The p53 protein expression indicates a stress cell response. Decrease in p53 protein can cause a decrease in the mechanism of apoptotic cells.

CONCLUSION

The dose of gondomono extract, the higher the expression of p53 protein, the more likely it is to increase, which indicates an apoptotic process. Likewise, the higher the dose of gondomono extract, the higher the expression of p53 protein, the higher. Gondomono extract as a cancer cell killer requires a minimum dose of 368.51 ppm.

REFERENCES

1. Alison MR. Cancer. Nat Publ Gr. 2001.
2. Jemal A, Bray F, Ferlay J. Global Cancer Statistics: 2011. CA Cancer J Clin. 1999;49(2):1,33-64.
3. Heron M. Deaths: Leading Causes for 2008. Natl Vital Stat Rep. 2012;60(6):1–94.
4. Kemenkes RI. Riset Kesehatan Dasar 2013. Jakarta: Kemenkes RI; 2013.
5. World Health Organization. Policy and Advocacy (Cancer Control: Knowledge into Action: WHO Guide for Effective Programmes: Module 6). Geneva: WHO; 2008.
6. Donaldson MS. Nutrition and Cancer: A Review of the Evidence for an Anti-cancer Diet. Nutr J. 2004;3:1-21.
7. Wetwitayaklung P, Phaechamud T, Keokitichai S. The Antioxidant Activity of *Caesalpinia sappan* l. Heartwood in Various Ages. Naresuan Univ J. 2005;13(2):43-52.
8. Pérez-Amador MC, Ocotero VM, Castañeda JMG, Esquinca ARG. Alkaloids in *Solanum torvum* Sw (*Solanaceae*). Phyton (B Aires). 2007;76:39-45.
9. Narwanto MI, Rahayu M, Soeharto S, Nurdiana N, Widodo MA. Identification and in Silico Analysis of Anti Inflammation and Anti Oxidant Potentials of Polyphenol Compounds in Methanol Extract of *Tamarindus indica* Seeds. J Agromedicine Med Sci. 2018;4(1):13.
10. Yuhendri, Jasril, Yuharmen. Isolasi dan Uji Toksisitas Metabolit Sekunder Ekstrak n-Heksana Kulit Batang Tumbuhan Polyalthia pulchra var. angustifolia King (*Annonaceae*). Pekanbaru: FMIPA Univ Riau. 2013.
11. Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. J Immunol Methods. 1983;65(1-2):55-63.
12. Wahyuni S, Zakaria F, Witarto AB, Syah D, Suhartono M. Aktivitas Anti Kanker Senyawa-Senyawa Kitooligomer. Jurnal Teknologi dan Industri Pangan. 2006;17:12-22.
13. Abu-Dahab R, Afifi F. Antiproliferative Activity of Selected Medicinal Plants of Jordan against a Breast Adenocarcinoma Cell Line (MCF7). Sci Pharm. 2007;75(3):121-36.
14. Fakultas Farmasi UGM. Chemoprevention Research Center (CCRC). Yogyakarta: Fakultas Farmasi UGM.
15. Kwan YP, Saito T, Ibrahim D, Al-Hassan FMS, Ein Oon C, Chen Y, et al. Evaluation of the Cytotoxicity, Cell-cycle arrest, and Apoptotic Induction by *Euphorbia hirta* in MCF-7 Breast Cancer Cells. Pharm Biol. 2016;54(7):1223-36.