JKKI
SCIENTIA EST BASIC VITAE

Jurnal Kedokteran dan Kesehatan Indonesia

Indonesian Journal of Medicine and Health

Journal homepage: https://journal.uii.ac.id/JKKI

Cytotoxicity of ethanolic extract of fruit shells and seeds of Nyamplung (*Calophyllum inophyllum L.*) on WiDr colorectal cancer cells

Irfan Jaen Fathani*[1], Isnatin Miladiyah[2]
[1]Undergraduated student, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia
[2]Department of Pharmacology, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia

Original Article

ARTICLE INFO

Keywords:
Calophyllum inophyllum,
colorectal cancer,
cytotoxic,
IC_{50},
MTT assay

***Corresponding author:**
irfanjaenfathani@ymail.com

DOI: 10.20885/JKKI.Vol12.Iss2art10

History:
Received: May 3, 2021
Accepted: August 24, 2021
Online: August 31, 2021

ABSTRACT

Background: Colorectal cancer is the 3rd most common type of cancer in the world with unsatisfactory therapeutic effectiveness. One of the efforts being made to overcome this problem is by extracting various new chemotherapy agents, including herbal plants. The Nyamplung plant (*Calophyllum inophyllum L.*) is reported to contain calophyllolide, triterpene, coumarin, saponin, biflavonoids, benzophenones and neoflavanoids which are potential anticancer agents.

Objective: This study aims to determine the cytotoxic activity of ethanolic extract of fruit shells and seeds of Nyamplung (*Calophyllum inophyllum L.*) on WiDr colorectal cancer cells and their selectivity on normal cells (Vero).

Methods: The fruit and seeds of Nyamplung were extracted by maceration with 70% ethanol solvent. The cytotoxic test was carried out by the 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) method, with doxorubicin as a positive control. Data analyzed using Microsoft Excel software using linear regression analysis. The IC_{50} value and selectivity index were used as indicator of toxicity selectivity.

Results: The IC_{50} value of the ethanolic extract of Nyamplung fruit shells on WiDr was 42.47 µg/ml with a selectivity index of 1.50. Meanwhile, the IC_{50} value of ethanolic extract of Nyamplung seeds was 1,030.41 µg/ml with a selectivity index of 67,982,414.71. Doxorubicin as a positive control obtained IC_{50} value on WiDr cells of 3.49 µg/ml with a selectivity index of 764.41.

Conclusion: The ethanolic extract of Nyamplung seeds was not cytotoxic against WiDr colorectal cancer cells, while the ethanolic extract of Nyamplung fruit sell was moderate cytotoxic with low selectivity index.

Latar Belakang: *Kanker kolorektal merupakan jenis kanker terbanyak ketiga di dunia dengan efektivitas terapi yang belum memuaskan. Salah satu upaya yang dilakukan untuk mengatasinya adalah dengan penggalian berbagai agen kemoterapi baru, di antaranya dari tanaman herbal. Tanaman Nyamplung dilaporkan mengandung calophyllolide, triterpenoid, kumarin, saponin, biflavonoids, benzophenones dan neoflavanoids yang berpotensi sebagai zat antikanker.*

Tujuan: *Penelitian ini bertujuan untuk mengetahui aktivitas sitotoksik ekstrak etanol cangkang buah dan biji Nyamplung (Calophyllum inophyllum L.) terhadap sel kanker kolorektal WiDr dan selektivitasnya terhadap sel normal (Vero).*

Metode: *Buah dan biji Nyamplung diekstraksi secara maserasi dengan pelarut etanol 70%. Uji sitotoksik dilakukan dengan metode 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT), dengan doxorubicin sebagai kontrol positif. Analisis data dilakukan dengan perangkat lunak* Microsoft Excel *menggunakan analisis regresi linier. Nilai IC_{50} dan indeks selektivitas digunakan sebagai indikator toksisitas*

dan selektivitas.
Hasil: *Nilai IC_{50} ekstrak etanol cangkang buah Nyamplung pada sel WiDr sebesar 42.47 µg/ml dengan indeks selektivitas 1.50, sedangkan nilai IC_{50} ekstrak etanol biji Nyamplung sebesar 1.030,41 µg/ml dengan indeks selektivitas 67.982.414,71. Nilai IC_{50} doxorubicin sebagai kontrol positif pada sel WiDr yaitu 3,49 µg/ml dengan indeks selektivitas 764,41.*
Kesimpulan: *Ekstrak etanol biji Nyamplung tidak bersifat sitotoksik terhadap sel kanker kolorektal WiDr, sedangkan ekstrak etanol cangkang buah Nyamplung bersifat sitotoksik sedang dengan indeks selektivitas rendah.*

INTRODUCTION

Cancer cases in the world continue to increase. Based on GLOBOCAN 2020 data, 19,292,789 new cancer cases were recorded from various parts of the world and 9,958,133 of them died in 2020. Colorectal cancer is the third most incident cancer in the world. About 1,931,590 new colorectal cancer was diagnosed in 2020.[1] The colorectal cancer is more incident 3–4 times among men than women and more common in developed nations than in developing nations. Age-standardized (world) incidence rates colorectal cancer per 100,000 population in both sexes is 19.5, in males is 23.4, and in females is 16.2. Age-standardized incidence rate among men is 30.1/100,000 in high-human development index (HDI) nations, while in low-HDI nations it is 8.4 (the same statistics for women are 20.9 and 5.9).[2] In 2020, colorectal cancer is the second most deadly cancer in the world with about 935,173 deaths. Age-standardized (world) mortality rates per 100,000 of colorectal cancer in both sexes is 9.0. Colorectal cancer also an emerging public health problem in Indonesia. In 2020, the age-standardized incidence rates of colorectal cancer per 100,000 populations in Indonesia were 6.7 for both sexes. According to these data, colorectal cancer is cancer that must be of concern to the world and Indonesian health sector since it is included in the top three cancers, both in terms of incidence and mortality.[1]

Colorectal cancer is a malignancy originating from the large intestine tissue consisting of the colon and/or rectum. This cancer can occur due to genetic inheritance, genetic mutations, or lesions in the colon due to infection.[3] Colorectal cancer is very rare in the 1950s but is currently among the top three most common cancers in the world. This occurs due to changes in the lifestyle of modern society which tends to be unhealthy, such as smoking, alcohol consumption, lack of exercise, or obesity.[4,5]

Colorectal cancer cases are usually treat by removing cancer tissue for an early stage of cancer, using chemotherapy for palliative treatment, and using radiotherapy. The use of chemotherapy is considered poor due to side effects, such as a lack of selectivity against malignant cells and high prices. It is necessary to look for alternative therapies with lower side effects, for example, herbal medicines. The use of herbal medicines along with chemotherapy is reported to reduce side effects and increase the effectiveness of therapy.[6] Research on anticancer substances obtained from plants has been widely carried out. *Calophyllum inophyllum L*. is a plant that has the potential as an anticancer.[5]

Calophyllum inophyllum L. or "Nyamplung" contain calophyllolide, triterpene, coumarin, biflavonoids, benzophenones, and neoflavanoids which are potential anticancer agents.[7] Previous studies had shown that the content of these substances can produce cytotoxic or anti-proliferative effects on human cancer cells. In previous studies, it was found that Nyamplung fruit extract had a cytotoxic effect on MCF-7 cancer cells. Nyamplung fruit extract induces apoptosis through activation of caspase-3 and inhibits the formation of reactive oxygen species (ROS) in MCF-7 breast cancer cells.[8] The stems and leaves have also been shown to have a growth inhibitory effect on the HL-60 leukemia cells.[9] The oil from Nyamplung seeds also has the power to heal wounds by increasing cell proliferation, collagen production, and glycosaminoglycans.[10] Apart from being used in the health sector, Nyamplung seeds can also be used as biodiesel fuel.[11] Currently, testing the cytotoxic activity of Nyamplung fruit on

colorectal cancer cells has not been carried out. Therefore, a study on the cytotoxic test of the fruit shells and seeds of Nyamplung (*Calophyllum inophyllum L.*) on WiDr colorectal cancer cells and normal Vero cells is necessary. This study is expected to be the basis for further study in exploring the potential of the Nyamplung plant as an alternative to existing anticancer drugs.

METHODS

Research design

This was a laboratory experimental study a laboratory experimental study with a post-test-only control group design. The study was conducted from July 2020 to September 2020. The subjects of this study were WiDr cancer cells and normal Vero cells obtained from the Laboratory of Parasitology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia.

Collection and preparation of Nyamplung shell and seed ethanol extract

The fruit shells and seeds of the Nyamplung were obtained from the Qiara Herbal Collection shop (West Jakarta, Indonesia). The sample determination test was carried out at the Laboratory of Pharmaceutical Biology, UGM, Yogyakarta, Indonesia. The extraction process was carried out at the Laboratory of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia (UII), Yogyakarta, Indonesia. Extraction was carried out by maceration method using 70% ethanol. The Nyamplung fruit was broken down in advance to separate the seeds and the fruit shells. A total of 1 kg of seeds and 1 kg of Nyamplung fruit shells were washed under flowing water, dried, and then put in a cabinet dryer with a temperature of 50oC for 2 hours. The results of the drying process run well with the use of a grinder to make it powder, then macerated with 70% ethanol for 24 hours. The maceration results were filtered with a Buchner funnel to obtain macerate. Furthermore, the macerate was put into a rotary evaporator to obtain the extract from the seeds and fruit shells of the Nyamplung separately. The extract was evaporated on a water bath to maximize the evaporation of the remaining solvent.

Preparation of sample

The ethanolic extract of the Nyamplung fruit which was divided into two forms of seed and fruit shell samples were made separately. The stock sample solutions (seed and fruit shells) were prepared with a concentration of 100,000 µg/ml. Dissolve 21.5 mg of ethanolic extract of Nyamplung seeds in 215 µl dimethyl sulfoxide (DMSO) and 14 mg of ethanolic extract of Nyamplung fruit shells were dissolved in 140 µl DMSO then added with RPMI 1640 (for WiDr cells) or Dulbecco's modified eagle medium (DMEM media for Vero cells). The stock solution was put into a closed sterile microtube, and stored in the refrigerator. The standard drug doxorubicin was readily available in concentrations of 2,000 µg/ml. After that, serial production of the test solution concentration was carried out using the multilevel dilution method. The ethanolic extract of the seeds and the fruit shell of the Nyamplung were each made in 6 concentrations, namely 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml. The concentration was made twice each for treatment on WiDr cells and Vero cells. Doxorubicin as a positive control was made with 6 concentrations, 20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, and 0.625 µg/ml.

Cytotoxic activity assays

WiDr and Vero cells were cultured until cell harvesting and cell planting were carried out on a 96-well microplate. Two microplates were used, one for WiDr cells, and one for Vero cells. Positive control was a group of cells given doxorubicin. Control cell contained cells and culture media. Control media contained culture media only without cells. The control wells of media and cells were added 100 µl of media according to each cell. Furthermore, each well was added 100 µl of the concentration

series that had been made according to the distribution that had been determined in 96 well plates. Cells that had been treated were incubated at 37°C for 24 hours, after which each well was given 100 µL of 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide reagent (MTT reagent). The microplate was incubated for 4 hours in an incubator at 37°C, then the condition of the cells was checked with an inverted microscope. If the formazan had formed, a stopper solution of 100 µL was added, namely Sodium dodecyl sulfate 10% (SDS 10%) in 0.01 N HCl. The results were then observed using an Enzyme-linked immunosorbent assay (ELISA) reader at a wavelength of 550-600 nm.

Measurement of cytotoxic activity and selectivity index

Data analysis from the cytotoxic test was carried out to calculate the IC_{50} value by linear regression analysis. The first step was to calculate the percentage of living cells using the following formula:

$$\% \text{ living cells} = \left(\frac{\text{Absorbance of treatment} - \text{absorbance of media control}}{\text{absorbance of cell control} - \text{absorbance of media control}}\right) \times 100\%$$

Furthermore, a log graph of the concentration vs the percentage of living cells was made with a scatter type chart. Then, the linear regression equation was seen by displaying the trendline. Then, y = 50% was entered to get the x value. The antilog of x was calculated to get the IC_{50} value. Next, the selectivity index was calculated using the following formula:

$$\text{Selectivity index (SI)} = \frac{(IC_{50} \text{ Vero Cell})}{(IC_{50} \text{ WiDr Cell})}$$

This study has received ethical approval from the Health and Research Ethics Committee of the Faculty of Medicine UII, Yogyakarta, Indonesia with letter number 3/Ka. Kom. Et/70/KE/VII/2020.

RESULTS

Based on the results of the determination test at the Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM, Yogyakarta, Indonesia the sample was genuine and true *Calophyllum inophyllum L*. The extraction results were in two different shapes for shells and seeds. In the extraction of 1 kg shell and skin, 12.853 grams of thick extract were produced while in the extraction of 1 kg of seeds, 190 grams of oil and sediment were produced.

Dehydrogenase enzyme in cell mitochondria might change the water-soluble yellow MTT to the purple, water-insoluble formazan. Thus, the purple color indicates the number of cells that are still alive because the dehydrogenase enzyme is still functioning. The purple color due to formazan crystals shows the number of living cells, as presented in Figure 1. The absorbance measurement results of each variable can be seen in Tables 1, 2, and 3.

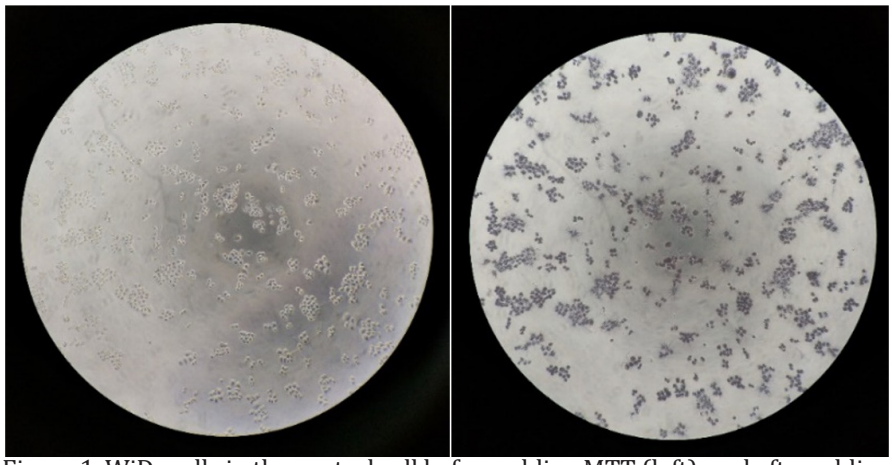

Figure 1. WiDr cells in the control cell before adding MTT (left), and after adding MTT (right)

Table 1. Measurement of the absorbance of Nyamplung fruit extract on WiDr cells

Concentration (µg/ml)	Concentration Log	Nyamplung seed extract			Nyamplung fruit shell extract		
		Absorbance mean	Cell viability (%)	Cell death (%)	Absorbance mean	Cell viability (%)	Cell death (%)
200	2.30	0.43	0.43	39.56	0.14	-0.20	100.20
100	2	0.60	0.60	6.09	0.14	0.68	99.31
50	1.69	0.67	0.67	-7.80	0.44	60.84	39.15
25	1.39	0.70	0.70	-15.26	0.51	75.08	24.91
12.5	1.09	0.73	0.73	-21.42	0.59	92.60	7.39
6.25	0.79	0.72	0.72	-18.54	0.67	108.89	-8.89
Cell Control	-	0.63	0.63	-	0.63	-	-
Cell Media	-	0.14	0.14	-	0.14	-	-

Table 2. Measurement of the absorbance of Nyamplung fruit extract on Vero cells

Concentration (µg/ml)	Concentration Log	Nyamplung seed extract			Nyamplung fruit shell extract		
		Absorbance mean	Cell viability (%)	Cell death (%)	Absorbance mean	Cell viability (%)	Cell death (%)
200	2.30	0.63	87.40	12.59	0.13	9.67	90.32
100	2	0.69	96.04	3.95	0.24	26.17	73.82
50	1.69	0.70	97.19	2.81	0.56	76.79	23.20
25	1.39	0.69	96.56	3.43	0.67	93.13	6.86
12,5	1.09	0.69	96.15	3.85	0.68	94.79	5.20
6,25	0.79	0.70	97.71	2.28	0.66	92.04	7.96
Cell Control	-	0.71	-	-	0.71	-	-
Cell Media	-	0.07	-	-	0.07	-	-

Table 3. Measurement of the absorbance of doxorubicin on WiDr cells and Vero cells

Concentration (µg/ml)	Concentration Log	WiDr Cells			Vero Cells		
		Absorbance mean	Cell viability (%)	Cell death (%)	Absorbance mean	Cell viability (%)	Cell death (%)
20	1.30	0.26	19.33	80.66	0.49	65.40	34.59
10	1	0.35	37.41	62.58	0.52	69.71	30.28
5	0.69	0.37	40.86	59.13	0.53	71.54	28.46
2.5	0.39	0.41	48.27	51.72	0.58	79.76	20.23
1.25	0.09	0.49	64.57	35.43	0.57	76.84	23.15
0.625	-0.20	0.61	88.41	11.58	0.56	76.53	23.46
Cell Control	-	0.67	-	-	0.71	-	-
Cell Media	-	0.07	-	-	0.07	-	-

Table 4 shows the results of the line equation until IC_{50} and selectivity index results were obtained. According to the US National Cancer Institute (NCI), there are four categories of toxic compounds seen from their IC_{50} namely $IC_{50} \leq$ 20 µg/ml (high), IC_{50} 21-200 µg/ml (moderate), IC_{50} 201-500 µg/ml (low), and $IC_{50} >$ 501 µg/ml (non-toxic).[12] The selectivity index value shows the level of safety of the extract against cancer cells and normal cells, which was calculated by comparing the IC_{50} value of the extract on normal cells (Vero cells) and the extract IC_{50} on WiDr colorectal cancer cells. Extracts are said to have high selectivity if the selectivity index value is > 2.[13]

Table 4. Calculation of the IC_{50} value of Nyamplung fruit extract and doxorubicin and their selectivity index

Material	WiDR Cells		Vero Cells		Selectivity Index
	Line Equation	IC_{50} Value	Line Equation	IC_{50} Value	
Ethanolic extract of the Nyamplung seeds	y= -36.12x + 158.83	1,030.41	y= -4.85x + 102.7	70,049,759.94	67,982,414
Ethanolic extract of Nyamplung fruit shells	y= -79.301x + 179.11	42.47	y= -60.17x + 158.62	63,828	1.50
Doxorubicin	y= -41.215x + 72.417	3.49	y= -8.09x + 77.73	2,674.44	764,416

DISCUSSION

Nyamplung fruit consists of seeds and shells. According to Kartika et al. , the seeds of Nyamplung contain abundant oil. Therefore, when the extraction was carried out, it produced the form of oil.[14] A thick extract was obtained from the shell extraction. Ethanol is a polar solvent that can attract polar compounds from natural substances. The shells and seeds of Nyamplung are known to have polar toxic potential compounds, such as triterpenoids, calophyllolides, coumarin, flavonoids, saponins, and resins.[7,11,14]

In the case of colorectal cancer, there is an increase in the enzyme topoisomerase II.[15] Doxorubicin has a mechanism of inhibiting the topoisomerase II enzyme which results in deoxyribonucleic acid (DNA) damage and cell apoptosis.[16] Doxorubicin is broad-spectrum cancer chemotherapy that has good efficiency and is widely used in testing for colorectal cancer.[17] Doxorubicin is a cancer drug that is quite toxic to WiDr colon cancer cells.[18] Doxorubicin can be used as a positive control in the colorectal cancer model since it can induce the apoptotic process.[19] A previous study used WiDr cells concluded that doxorubicin has an IC_{50} of 9.22 µg/mL against WiDr cells indicating that it is toxic.[20] According to Wijaya, doxorubicin has an IC_{50} of 5.22 µg/mL against WiDr cells.[21] In this study, the IC_{50} positive control results of doxorubicin 3.49 µg/mL were of toxic value for WiDr cells with a good selectivity index of 764.416.

The cytotoxic potential of the ethanolic extract of Nyamplung fruit shells on WiDr cells was classified as moderate, with IC_{50} of 42.47µg/ml. There may be saponin and triterpenoid compounds in the shell of the fruit of the Nyamplung.[14,22] Saponin compounds are one of the anticancer compounds that can be obtained from nature. There are various studies regarding the cytotoxic mechanism of saponin compounds. Those studies concluded that saponins cause cell death effects by apoptosis or non-apoptosis. The most widely reported apoptotic mechanism is the intrinsic pathway of the apoptotic mechanism. Non-apoptotic mechanisms include reduced NO production, cytoskeleton disintegration, and stimulation of cell death by autophagy.[23] Triterpenoids are known to have anti-cancer properties through

the preventive mechanisms of activation of nuclear factor-kappa B and induction of apoptosis.[24] Although the ethanolic extract of Nyamplung fruit shells has moderate levels of toxicity in WiDr cells, it also has moderate levels of toxicity in Vero cells. Therefore, the results obtained a low selectivity index of 1.50. This indicates that the ethanolic extract of the Nyamplung fruit shell is not safe for normal cells.

The ethanolic extract of Nyamplung seeds obtained IC_{50} of 1.030,41 µg/ml in WiDr cells and 70,049,759.94 µg/ml in Vero cells. These results concluded that the ethanolic extract of Nyamplung seeds did not have a cytotoxic effect on WiDr cells and Vero cells. This is different from the study conducted by Hsieh et al. which found that Nyamplung seeds with methanol solvent had properties to inhibit the proliferation of DLD-1 colon cancer cells.[25] Nyamplung seeds contain many flavonoids, triterpenoids, and xanthone compounds.[26] Compared to ethanol solvent, methanol solvent can filter the active ingredients of flavonoids, triterpenoids, alkaloids, and phenolic compounds better.[27] This explains the use of methanol extract from Nyamplung seeds gave better cytotoxic results than the ethanol solvent in this study. The solvents used in this study most likely influenced the IC_{50} results.

The limitation of this study is that it did not examine the substance content of Nyamplung fruit and was also limited to WiDr cells. Thus, it is necessary to carry out further study in the form of analysis of substance content in Nyamplung fruit and also cytotoxic tests on other cancer cell lines. Moreover, it is also necessary to extract using a solvent other than ethanol to determine the best cytotoxic level against WiDr cells and to have a good selectivity index.

CONCLUSION

The ethanolic extract of Nyamplung seed has not been proven to be effective on WiDr colorectal cancer cells since it does not have cytotoxic activity. Meanwhile, ethanolic extract of Nyamplung fruit shells has moderate cytotoxic but has a low selectivity index. Further study needs to be done to maximize its cytotoxic potential and selectivity in the field of anticancer herbal medicine.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

ACKNOWLEDGEMENT

The authors would like to thank to Faculty of Medicine, UII, Yogyakarta, Indonesia for funding this study through the *Hibah Penelitian Mahasiswa*. The authors also thanks to technicians from Laboratory of Parasitology, Faculty of Medicine, Public Health, and Nursing, UGM, Yogyakarta, Indonesia and Laboratory of Pharmacy, Faculty of Mathematics and Natural Sciences, UII, Yogyakarta, Indonesia for guided and accompanied in the laboratory.

REFERENCES

1. The Global Cancer Observatory. Cancer fact sheet. World Health Organization. 2019.
2. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Przeglad Gastroenterologiczny. 2019;14(2):89–103.
3. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJH WT. Colorectal Cancer.
4. Kusuma AW, Aries Nurulita N, Hartanti D. Efek sitotoksik dan antiproliferatif kuersetin pada sel kanker kolon WiDr.Pharmacy. 2010;7(3):107-122.
5. Kementerian Kesehatan. Panduan Penatalaksanaan kanker kolorektal. Kementerian Kesehatan Republik Indonesia. 2016. 76 p.
6. Fu B, Wang N, Tan HY, Li S, Cheung F, Feng Y. Multi-component herbal products in the prevention and treatment of chemotherapy-associated toxicity and side effects: A review on experimental and clinical evidences. Frontiers in Pharmacology. 2018;9:1394.
7. Ragasa CY, Ebajo V, Reyes MMDL, Mandia EH, Brkljača R US. Triterpenes from Calophyllum inophyllum linn. International

Journal of Pharmacognosy and Phytochemical Research. 2015;7(4):718–22.
8. Shanmugapriya, Chen Y, Kanwar JR, Sasidharan S. Anticancer activity and molecular mechanism of polyphenol rich Calophyllum inophyllum fruit extract in MCF-7 breast cancer cells. Nutrition and Cancer. 2017;69(8):1308–24.
9. Li YZ, Li ZL, Yin SL, Shi G, Liu MS, Jing YK, et al. Triterpenoids from Calophyllum inophyllum and their growth inhibitory effects on human leukemia HL-60 cells. Fitoterapia. 2010;81(6):586–9.
10. Ansel JL, Lupo E, Mijouin L, Guillot S, Butaud JF, Ho R, et al. Biological activity of polynesian Calophyllum inophyllum oil extract on human skin cells. Planta Medica. 2016;82(11–12):961–6.
11. Leksono B, Windyarini E, Hasnah TM. Budidaya Nyamplung (Calophyllum inophyllum L.) untuk bioenergi dan prospek pemanfaatan lainnya. IPB Press. 2014. 4–5 p.
12. Sajjadi SE, Ghanadian M, Haghighi M, Mouhebat L. Cytotoxic effect of Cousinia verbascifolia Bunge against OVCAR-3 and HT-29 cancer cells. Journal of HerbMed Pharmacology. 2015;4(1):15–9.
13. Demirgan R, Karagöz A, Pekmez M, Önay-Uçar E, Artun FT, Gürer Ç, et al. In vitro anticancer activity and cytotoxicity of some papaver alkaloids on cancer and normal cell lines. African Journal of Traditional, Complementary and Alternative Medicines. 2016;13(3):22–6.
14. Kartika IA, Cerny M, Vandenbossche V, Rigal L, Sablayrolles C, Vialle C, et al. Direct Calophyllum oil extraction and resin separation with a binary solvent of n-hexane and methanol mixture. Fuel. 2018;221:159–64.
15. Hendricks A, Gieseler F, Nazzal S, Bräsen JH, Lucius R, Sipos B, et al. Prognostic relevance of topoisomerase II α and minichromosome maintenance protein 6 expression in colorectal cancer. BMC Cancer. 2019;19(1):429.
16. Thorn Caroline F, Oshiro Connie, Marsh Sharon, Hernandez-Boussard Tina, McLeod Howard, Klein Teri E ARB. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenetics and genomics. 2011; 21(7):440-446.
17. Utami DT, Nugraheni N, Jenie RI, Meiyanto E. Co-treatment of Brazilein enhances cytotoxicity of Doxorubicin on WiDr colorectal cancer cells through cell cycle arrest. Indonesian Biomedical Journal. 2020;12(4):376–82.
18. Nuryastuti T, Pratiwi R, Puniawati Soesilo N. Efek Sitotoksik, antiproliferatif dan apoptosis serum tikus (Rattus novergicus Berkenhout, 1769) diberi Serbuk Tokek (Gekko gecko L.) per oral terhadap sel WiDR. Biogenesis: Jurnal Ilmiah Biologi. 2017;5(2):128–37.
19. Rangkuti I. Y., Bestari R., Lubis A. I. Ethyl acetate extract of Sabrang onion (E. Bulbosa (Mill.) Urb.) increases apoptosis of Widr cells. International Journal Of Scientific & Technology Research. 2020;9(2):5027–30.
20. Paputungan WA, Rotinsulu H YP. Standardisasi parameter spesifik dan uji aktivitas antikanker terhadap sel kanker kolon (Widr) dari ekstrak etanol Lamun (Enhalus Acoroides). Pharmacon. 2017;6(3):189-199.
21. Wijaya L. Uji sitotoksik ekstrak etanol kulit kayu manis (cinnamomum burmanni nees & t. Nees.) terhadap sel kanker kolon. Skripsi thesis: Universitas Setia Budi Surakarta. 2015.
22. Santi S. Penelusuran senyawa sitotoksik pada kulit biji Nyamplung (Calophyllum inophyllum L.) dan kemungkinan korelasinya sebagai antikanker. Jurnal Kimia. 2009;3(2):101-108.
23. Podolak I, Galanty A, Sobolewska D. Saponins as cytotoxic agents: A review. Phytochemistry Reviews. 2010;9(3):425–74.
24. Petronellia A, Pannitterib G, Testaa U. Triterpenoids as new promising anticancer drugs. Anti-Cancer Drugs. 2009;20(10):880–92.
25. Hsieh C, Lin YW, Chen CH, Ku W, Ma F, Yu H, et al. Yellow and green pigments from Ca-

lophyllum inophyllum L. Seed oil induce cell death in colon and lung cancer cells. Oncology Letters. 2018;15(4):5915–23.
26. Ginigini J, Lecellier GJ, Nicolas M, Nour M, Hnawia E, Lebouvier N, et al. Chemodiversity of Calophyllum inophyllum L. oil bioactive components related to their specific geographical distribution in the South Pacific region. PeerJ. 2019;2019(5).
27. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of severinia buxifolia. Journal of Food Quality. 2019;1:1-9.