



The Ethanolic Extract of Annonaceous sp. to Inhibit Vascular Endothelial Growth Factor (VEGF) as Anticancer Modalities

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ABSTRACT

Background: Cancer has become a major public health problem worldwide, with breast cancer is the most common cancer among women and one of the most important causes of death among them. Management of breast cancer have some adverse effects, with many factorx=s shiouln be considered to ordered the therapy. Use of chemotherapy will suppress cell proliferation and trigger apoptosis, but it cannot just focus on cancer cells only, but also would affected on other cells and cause. **Objective:** This study aim to analyze the inhibition effect of the Annonaceous sp. ethanolic extract to Vascular Endothelial Growth Factor (VEGF) on MCF-7 cells line **Material and Methods:** This study is an analytic experimental study used Randomized Complete Block Design (RCBD) with three repetitions. The extract was dissolved in 1 ml of DMSO with a concentration of 0.1%. Furthermore, dilution was made with a dose of 0 µg / ml (K); 25 µg / ml (P1); 50 µg / ml (P2); 100 µg / ml (P3); 200 µg / ml (P4). MCF-7 cells line were cultured used RPMI 1640 Medium with 80 – 90% confluent. The ethanolic extract of Annonaceous sp. were exposed to MCF-7 cells for 48 hours. Analyze of VEGF level use ELISA Methods with λ=405 nm. **Results:** The results of ELISA analyze shows that the ethanolic extract of Annonaceous sp. have potential effect to decreased of VEGF expression on sample with treatment on some concentration (p < 0,05). **Conclusion:** The ethanolic extract of Annonaceous sp. has shows the potential effect to decreased of VEGF level on MCF-7 cells line

1. Introduction

Cancer has become a major public health problem worldwide cause of continuously increased of prevalence over years and also is one of the main causes of mortality worldwide. International Agency for Research on Cancer (IARC) in 2018 reported there were 18.1 million new cancer sufferers, with mortality rate was 9.6 million.^{1,2}

Breast cancer is the most common cancer among women and one of the most important causes of death among them. WHO reported breast cancer incidence in 2018 is the most frequent diagnosed cancer among women, impacting 24% of all cases (2.1 million patients) with mortality rate were 15% of total.³ In Indonesia, breast cancer is the most frequently

diagnosed cancer by 18,6% of relative frequency. While breast cancer rates are higher among women in more developed regions, rates are increasing in nearly every region globally.^{4,5}

Systemic therapy of breast cancer has been conducted for the last hundred years since Beatson in 1896 demonstrated that ovarium ablation changes hormonal balancing in woman with breast cancer. Breast cancer management is determined by the extent, stage and expression of the biomolecular or biomolecular-signaling agent. Moreover, the choice of chemotherapy or hormonal therapy for breast cancer patients depends on technological advancement such as immunohistochemistry and biomolecular assay.^{4,6}

Management of breast cancer also has some adverse effects. In addition, factors of age, co-morbid disease, evidence-based study, cost effective utility, and when to stop the systemic treatment should be considered. Chemotherapy is a treatment using anticancer compounds by systemic used to suppress cell proliferation and trigger apoptosis. So that in use, chemotherapy cannot just focus on cancer cells, but also would be affected on other cells and cause decreased immune function as a result of adverse effects.⁷

For many years traditional herb medicines have been used as medical treatment, especially in developing country. Plants have been used in medicine for their natural antiseptic properties. Studies to developing the potential activity compound and the uses of plants for the preparation of potential drugs including cancer. There are about 40.000 plants in the world and 30.000 have been reported founded in Indonesia. And 940 of them have utilized as a traditional medicine. Medicinal herbs and their derivate phytochemicals are being increasingly recognized as useful complementary treatments for cancer. Currently, several plant-derived compounds have been successfully used as cancer therapy, such as vincristine and vinblastine isolated from *Catharanthus roseus*. Several study of phytochemicals compound shows that antioxidant and anti-inflammatory properties can inhibit tumor initiation, promotion and progression. The most important in studying to developing herbs as anticancer referring to a standard quality, efficacy and safety. Therefore, we need an analysis against the mechanism of herb on molecular level.^{8,9,10}

Acetogenins (ACG) are naturally occurring compounds that are chemically one of the least investigated families. ACG are known to be very potent cytotoxic compounds, targeting the reduced nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase (also known as complex I) which is a membrane bound protein of the mitochondrial electron transport system, and the ubiquinone linked NADH oxidase in the plasma membrane of cancerous cells. Inhibition by these mechanisms results in adenosine triphosphate (ATP)

deprivation, which leads to apoptosis of the highly energy demanding tumor cells. The acetogenins are now considered as the most potent (effective in nanomolar concentrations) known inhibitors of the mitochondrial complex I. More recently the annonaceous acetogenins have also been shown to overcome resistance in multidrug resistant (MDR) tumors.^{11,12}

Angiogenesis is an essential step for breast cancer progression and dissemination. The development of new blood vessels in cancer setting (angiogenesis) is conducted by numerous physiological and pathological stimuli, where the main stimulus is hypoxia. Molecular players of angiogenesis have been characterized since the early years of angiogenic studies, and one of the most prominent stimulating growing factors is certainly the vascular endothelial growth factor family. The most prominent member of this family, vascular endothelial growth factor (VEGF, VEGF-A) is the foremost controller of physiological and pathological angiogenesis. Accordingly, numerous VEGF inhibitors have been approved by the North American Food and Drug Administration (FDA) for the treatment of advanced cancer and neovascularisation related to the macular degeneration.^{13,14}

2. Methods and Material

This study was conducted on May – September 2020 at Laboratorium of Cell Culture and Cytogenetic of Padjajaran University, Bandung. This study used a Randomized Complete Block Design (RCBD) with three repetitions. MCF-7 cells line used in this study were from *American Type Cultural Collection* (ATCC) and cultured used RPMI 1640 Medium with 80 – 90% confluent.

Cell culture

The cells were culture by Roswell Park Memorial Institute Medium (RPMI) 1640. This media supplemented with 10% Fetal Bovin Serum (FBS) Gibco™ (Thermo Fisher Scientific Cat.26140-079) and 0,2 mL bovine insulin (Sigma Aldrich Cat. No. 15500 and CAS RN 11070-73-8) at 37° C in 5% CO₂. Thawing process performed in waterbath at 37° C for

2-4 min. Then, 5×10^4 cell was taken into T-flask and incubated at 37° C in CO₂ 5%. When cells density reached 80% confluent, the trypsinization was done using 0,25% trypsin+0,53mM EDTA solution and then subcultured into new culture vessels, also incubated at 37° C in CO₂ 5%. After two times passaging, the MCF-7 cells ready to be treated.

The ethanolic extract of Annonaceous were exposed to MCF-7 cells line while confluent of medium was 80-90%. The extract was dissolved in 1 ml of DMSO with a concentration of 0.1%. Furthermore, dilution was made with a dose of 0 µg / ml (K); 25 µg / ml (P1); 50 µg / ml (P2); 100 µg / ml (P3); 200 µg / ml (P4). Cells were incubated for 48 hours after treatment. Supernatant after 48 hours incubation were incubation in microwell coated by VEGF antibodies and incubation for 1 hour. After the incubation, sample were given of Horse Radish Peroxidase (HRP) and incubated 30 minutes more. The sample were added by TMB and sample ready to

analyze used ELISA methods with $\lambda=405$ nm.

Statistical analysis

Comparison of mean values between treatment are presented as mean±SD and analyzed using ANOVA followed by LSD test with a 95% confidence level.

3. Result

The results of ELISA analyze shows that any decreased of VEGF expression on sample with treatment on some concentration ethanolic extract of Annonaceous. Table 1 show the result of VEGF expression analyze with 1 control (0 ng/mL) and 4 sample with different concentration (0.1 ng/mL, 0,5 ng/mL, 1 ng/mL, dan 5 ng/mL). The result show by 0,5 ng/mL, 1 ng/mL, and 5 ng/mL concentration of ethanolic extract of Annonaceous have decreasing VEGF expression ($p < 0,05$).

Table 1. VEGF Level Pre and Post Hours Treatment

| Concentration | VEGF (Mean ± SD) | P value |
|---------------|------------------|---------|
| 0 ng/mL | 3.55 ± 0.84 | |
| 0.1 ng/mL | 3.09 ± 0.01 | 0.06 |
| 0.5 ng/mL | 2.66 ± 0.13 | 0.01 |
| 1 ng/mL | 1.05 ± 0.24 | 0.007 |
| 5 ng/mL | 0.66 ± 0.57 | 0.001 |

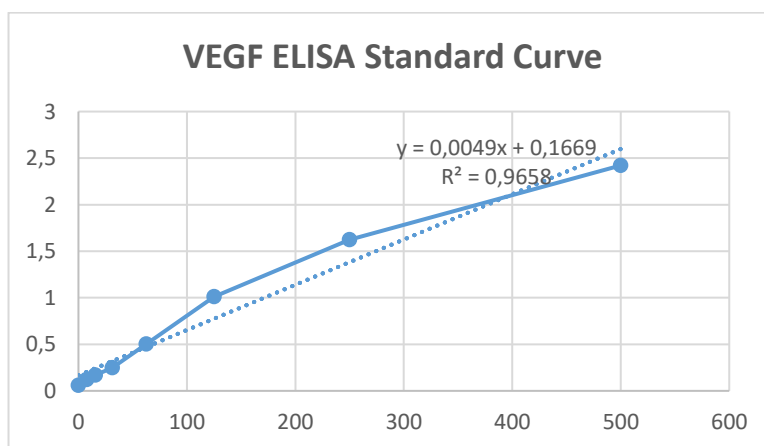


Table 1. VEGF Standard Absorbance Curve by ELISA Methods

4. Discussion

In generally, anticancer agents have a narrow therapeutic window, therefore it is needed to analyze

on cellular-level to developing an anticancer agent in order to determine the lowest concentrations that have therapeutic effects. Vascular endothelial growth factor

(VEGF, VEGF-A) is a major regulator of physiological and pathological angiogenesis. The observation that tumor growth can be accompanied by increased vascularity was reported more than a century ago.^{14,15}

Angiogenesis is an essential step for breast cancer progression and dissemination. The development of new blood vessels in cancer setting (angiogenesis) is conducted by numerous physiological and pathological stimuli, where the main stimulus is hypoxia.¹³ Acetogenin has known the potential effect as an anticancer agent. Our previous research shows that these compounds can trigger apoptosis and inhibit the proliferation of MCF-7 breast cancer line cells.¹⁶

The results of this study showed that administration of the ethanolic extract of Annonaceous for 48 hours was able to reduce VEGF levels of breast cancer cells MCF-7 cells line by significantly in some different concentration (Table 1).

Acetogenins (ACG) are naturally occurring compounds that are chemically one of the least investigated families. Mangal et al, (2016) have observed that the relative potency of acetogenins can be categorized as adjacent bis-THF ACGs > nonadjacent bis-THF ACGs > mono-THF ACGs > linear-THF ACGs. Among adjacent bis-THF ACGs, asiminocin (A100), asiminecin (A101), asiminacin (A102) and asimin (A103) are the most active compounds with in-vitro activity (ED50) in the range of 10⁻⁹ to 10⁻¹² µg/mL.¹⁷

In other study used Pawpaw crude extract (CE) and purified acetogenins showed inhibition effect to hypoxia-inducible factor-1 (HIF-1)-mediated hypoxic signaling pathways in tumor cells. In T47D cells, pawpaw CE and the acetogenins 10-hydroxyglaucanetin (**1**), annonacin (**2**), and annonacin A (**3**) inhibited hypoxia-induced HIF-1 activation with IC₅₀ values of 0.02 µg/mL, 12 nM, 13 nM, and 31 nM, respectively. This inhibition correlates with the suppression of the hypoxic induction of HIF-1 target genes *VEGF* and *GLUT-1*. The induction of secreted VEGF protein represents a key event in hypoxia-induced tumor angiogenesis. Both the extract and the purified acetogenins blocked the angiogenesis-

stimulating activity of hypoxic T47D cells *in vitro*. Pawpaw extract and acetogenins inhibited HIF-1 activation by blocking the hypoxic induction of nuclear HIF-1α protein. The inhibition of HIF-1 activation was associated with the suppression of mitochondrial respiration at complex I.¹⁸

5. Conclusion

The ethanolic extract of Annonaceous has shows the potential to decreased of VEGF level on MCF-7 cells line. In addition, there are needed To investigate the decrease in secreted VEGF protein can translate into the suppression of angiogenesis by molecular study.

6. Acknowledgment

There is no conflict of interest in this study

7. References

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