

# The Alteration Level of VEGF of Colon Cancer Cell After Induction with Mandarin Orange (*Citrus Reticulata*) Peel Ethanol Extract

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**Abstract.** The natural ingredient-based therapy extracted from various plants is assumed more tolerable to cancer patients' bodies than synthetic drugs. To tell successful novel cancer therapy, an indicator is needed to confirm the result. VEGF is a pro-angiogenic factor that leads the cancer cells' invasion to another organ and is associated with high mortality in patients. Hence, the VEGF level is widely used as one of the successful cancer therapy indicators. Purposed of research elucidated the effect of Mandarin orange peel ethanol extract (EEKJM) to improve the reduction of VEGF levels in colon cancer in vitro. This study was an experimental posttest and control group design to analyze different level of VEGF on cancer cells at end of the study. Research began with the preparation of EEKJM followed by a cytotoxic extract test using the MTT assay method. Furthermore, the measurement of VEGF levels by the VEGF ELISA kit protocol. One Way ANOVA was performed to analyze data. Results showed the cytotoxic test of the EEKJM and doxorubicin against WiDr cells was 240 g/ml and 1.59 µg/ml. VEGF levels showed decreasing after being induced with EEKJM and doxorubicin with doses ½ IC50 and ¼ IC50. Conclusion: EEKJM was able to reduce VEGF levels and viability of WiDr colon cancer cells. The advantages are as much as initial evidence of the ability of the orange to treat cancer even though further animal studies or evidence of a pathway of decrease of the VEGF or other signals are required

**Keywords:** Cell culture; Citrus reticulata; colon cancer cell; cytotoxic test; VEGF.

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## INTRODUCTION

Cancer is detected as uncontrolled cell growth in the body, and this disease could lead to death if the cells spread out from the original region where they grow. In 2020, there were nearly 10 million deaths caused by cancer, dominated by breast, colorectal, lung, and cervix cancer. World Health Organization recorded that colorectal cancer becomes the second most common cancer to cause death with 916,000 fatalities, just behind lung cancer with 1.8 million (Riihimäki et al., 2016).

Cancer cells' ability to metastasize to other organs such as the thorax, nervous system, bone,

and peritoneum causes poor prognosis in colorectal cancer (De Palma et al., 2017). To move to another organ, cancer cells need a lot of nutrition, and this necessity is facilitated by blood vessels which could stimulate the process of angiogenesis (De Palma et al., 2017). The angiogenesis process is also stimulated by various inflammatory factors and the Vascular Endothelial Growth Factor (VEGF) is one of the tumor vascularization growth factors. VEGF's high expression indicates a high rate of metastasis, chemoresistance, and bad prognosis. Because of that, it makes sense that VEGF becomes the target of cancer therapy or medication.

There are many kinds of colorectal cancer

therapy, such as chemotherapy, surgery, and radio-therapy. Nevertheless, those kinds of therapies have some negative side effects on cancer patients (Schirrmacher, 2018). Radiotherapy itself is associated with long-term effects on anorectal function such as fecal incontinence. This phenomenon leads many scientists to develop alternative cancer therapy with herbal-based medicine, such as mushrooms (Prastiyanto et al., 2020) and even fruit, to overcome this problem. In this context, Indonesia is the second-largest orange imported country in ASEAN, especially Mandarin orange, because of its cheap price, sweet taste, and ease of getting in public stores. High consumption of Mandarin Oranges in Indonesia caused high peel waste also, so, this study was using Mandarin orange peel as a core material to reduce the waste by using it as alternative medicine. Mandarin Orange contains plenty number of active compounds, for example, flavonoids, alkaloids, terpene, and polyphenols (Wang et al., 2020).

Tangeretin belongs to flavonoid compounds that have anticancer potential (Raza et al., 2020). Tangeretin could block cancer cells' activity at the G1 phase, so it gives consequences to the normal cancer cell cycle. Mandarin Orange peel's tangeretin induces G1 arrest by increasing the expression of CDK-Inhibitors such as p21 protein in human colorectal carcinoma COLO 205 cells. Moreover, the orange peel's flavonoid is also known as a compound that can reduce the MMP (Matrix Metalloproteinase) level of colon cancer cells, therefore, the cancer cells are unable to metastasize to another part of the human body (Ademosun et al., 2015).

Besides the Mandarin Orange peel ethanol extract, this study also used doxorubicin as the positive control. Doxorubicin is an anthracycline antibiotic with antineoplastic activity and was widely used as chemotherapy medicine in many kinds of cancers (Rivankar, 2014). Doxorubicin compound is a congener hydroxylated derivate from daunorubicin. Anthracycline anticancer is an effective medicine and is used as a chemotherapy agent for cancer through multiple mechanisms (Van Der Zanden et al., 2021), but its use is limited because it has cardiotoxicity and other negative side effects.

WiDr cell is a human colon cancer cell that derives from the HT-29 cell colon cancer cell. This cell has a special characteristic, which is the high expression of COX-2 (Cyclooxygenase-2) which participates in colon cancer cells' growth and migration. Furthermore, WiDr is one kind of colon

cancer cell that has low sensitivity toward 5-fluorouracil (5-FU), the antimetabolite chemotherapy agent WiDr resistance to 5-FU is mediated by the increasing of thymidine synthase enzyme expression (the main target of 5-FU obstruction). Overall, the WiDr cell is suitable for the new compound screening model in colon cancer therapy.

In this research, mandarin orange (*Citrus reticulata*) extract was used because it has been proven to have anti-cancer activities, such as anti-proliferative, and reduce the cell migration in thyroid carcinoma cells (Celano et al., 2015). That could be a good sign for a further experiment using Citrus reticulata extract in anti-cancer activity such as colon cancer. So, the research purpose was to find out the potential effect of Mandarin orange peel ethanol extract (EEKJM) to improve the reduction of VEGF levels in WiDr colon cancer in vitro. This research is one of the sources of information about the benefit of Orange mandarine peel to reduce cancer cell progress by VEGF levels control at least in the cancer colon WiDr cell. This result also is a trigger to future research to explore another benefit of orange mandarin peel in the cancer field.

## METHODS

This study design was laboratory quantitative experimental with the post-test only control group. The population that was used in this research was WiDr colon cancer culture stock from Culture Cell Laboratory, Faculty of Health and Medical Science, Universitas Muhammadiyah Yogyakarta. This research had an Ethical Clearance number: 020/EC-EXEM-KEPK FKIK/2021.

### Mandarin Orange peel ethanol extract

Mandarin Orange peel ethanol extract was made with the maceration technique: the orange was washed with water flow and peeled. The peel then was thinly chopped and dried in the oven at 70 °C temperature for 5 days. After that certain time, the dried orange peel was blended with a blender until it became simplicia powder. This powder was added with 70% ethanol (1:4 for extract: ethanol) in the closed vessel for 5 days and stirred for each day. Liquid extract was obtained from this process, and the liquid was filtered in filter paper to form a macerate. A rotary evaporator was used to concentrate the macerate at 40°C temperature. The final step in this process was to evaporate the extract in a water bath until it became a thick extract and labeled (Astuti and

Primasari, 2020)

### Preparation of colon cancer cell culture

Colon cancer cell culture was using WiDr cancer cell as a model for colon cancer cells. Cell culture and thawing of cells were done by adding 4 ml complete medium after centrifuging the cell suspension for 5 minutes. The centrifugation outcome was then transferred into 2 dishes with 2 ml each, added by 5 ml complete medium, and kept in an incubator. When thawing cells were done, and the cell formed 80% confluent, then the WiDr cells were ready to harvest with trypsin-EDTA (trypsin 0.25%) in 3 minutes. The cell yields were then added to a 5 ml medium. WiDr cell count was done by taking 10 µl cell yields into the hemacytometer, with the calculation formula:

$$\frac{\text{Number of Cells in 4 chambers}}{4} \times 10^4$$

(Astuti, Y et al, 2021<sup>a</sup>)

### Microculture Tetrazolium Salt Assay (MTT Assay)

Microculture Tetrazolium Salt Assay (MTT Assay) was used to test the cytotoxicity in this study, with nine different groups. The first group is a negative control, which is WiDr colon cancer cells without any treatments. The second group is WiDr colon cancer cell with Doxorubicin treatment as a positive control with various doses (10 µg/ml; 5 µg/ml; 2.5 µg/ml; 1.25 µg/ml; 0.625 µg/ml; and 0.3125 µg/ml). The third, fourth, fifth, sixth, seventh, eighth, and ninth groups are WiDr colon cancer cells with Mandarin Orange ethanol extract in 6 different doses, which are 500 µg/ml; 250 µg/ml; 125 µg/ml; 62.5 µg/ml; 31.25 µg/ml; and 15.625 µg/ml. Positive and negative control groups which were given Mandarin Orange peel ethanol extract were performed in 3 repetitions in each treatment group. Finally, the data analysis of the cytotoxic test of Mandarin Orange peel ethanol extract was rated with cell viability, which used the formula:

$$\frac{(\text{treatments absorbantion} - \text{medium control absorbantion})}{(\text{control cell absorbantion} - \text{medium control absorbantion})} \times 100\%$$

(Astuti, Y et al, 2021<sup>b</sup>)

### Measurement of VEGF level

The measurement of VEGF level was done based on Manual Kit Human Vascular Endothelial Cell Growth Factor ELISA KIT Cat. No E008Hu protocol. The result of the VEGF level from the

ELISA reader was analyzed using SPSS 25 with normality test Shapiro-wilk (the number of samples <50), and then comparative study between doses was analyzed using one-way ANOVA.

## RESULTS AND DISCUSSION

Vasculature performs various roles, such as stimulating tumor growth, preserving the surrounding environment, providing invasive and growth signals, promoting metastasis, and producing systemic diseases associated with cancer. VEGF and the activation of the pathway have been identified in a large number of disease processes such as cancer due to the major angiogenic factors in the tumor and have a role in the early stages of tumor growth, metastasis, and progression (Z.-L. Liu et al., 2023; Yang & Cao, 2022). Several studies show that VEGF is involved in various stages of metastatic cascade and carcinogenesis (Ntellas et al., 2020).

The release of systemic and local factors during tumor growth causes phenotype diversity in the process of metastasis. While some new research has begun to characterize metastasis from a spatiotemporal or omics point of view, it remains difficult to capture the metastatic landscape correctly. The most dangerous aspect of cancer is its ability to spread. Measures in metastatic cascade are local invasions and intravasations, survival in the bloodstream, capture on distant organs and extravasation, and re-colonization (Ghalehbandi et al., 2023; Parveen et al., 2019). To treat cancer, numerous strategies have been developed to disrupt the VEGF-VEGFR signaling system (Apte et al., 2019; Elebiyo et al., 2022).

In contrast to traditional anticancer treatments, which aim to eradicate cancerous cells that are actively growing, anti-angiogenesis therapy aims to damage the blood vessels supplying tumors. According to this theory, the tumor's vascular regression could reduce the tumor to a microscopic, latent condition, transforming cancer from a deadly illness into a chronic, asymptomatic one (Wu et al., 2018).

Meanwhile, a lot of research has also explored the presence of active agents from plants and herbs to reduce the risk of cancer or tumor, whether it is cytotoxic to cancer cells or reduces the ability of metastatic cancer or tumor cells due to plants contains secondary metabolites like phenolics, flavonoids, alkaloids, lignans, and terpenoids (Henri et al., 2022; Kristiani & Kasmiyati, 2021). The many properties of plants'

secondary metabolites, such as their anti-cancer properties

As Astuti report that Soursop leaves had a potential effect on cytotoxic, antimigration, proapoptosis, and antiproliferative also reduced the number of E cadherin and VEGF on Widr cancer cell (Astuti et al., 2022; Astuti, Priambodo, Harimurti, et al., 2021; Astuti, Priambodo, Rahmah, et al., 2021; Astuti & Primasari, 2020). Taxus brevifolia Nutt contains of taxons as active antitumor agents in a mice model of oral squamous cell carcinoma statistically significant lower microvessel density and VEGF expression (Fakhri et al., 2021). Extract Ethanol Jeruk M mandarin able to reduce migration capacity of WiDr cancer cell (Astuti & Primasari, 2020). This research explored the potential of Extract Ethanol Jeruk M mandarin (EEKJM) on viability also anti VEGF on cancer cell line WiDr. As Table 1 showed. Toxicity test results of Mandarin Orange peel ethanol extract against colon cancer cell WiDr.

**Table 1.** Average of colon cancer cell WiDr viability against Mandarin Orange (*Citrus reticulata*) peel ethanol extract (EEKJM)

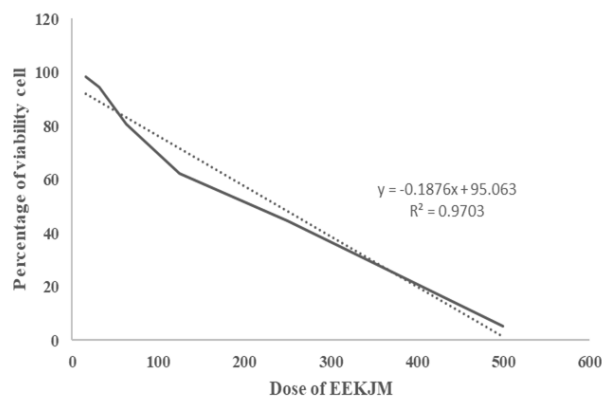
No	EEKJM Concentration (µg/ml)	Average of Cell Viability (%)
1.	500	5.12 ± 0.09
2.	250	44.74 ± 1.27
3.	125	62.43 ± 2.53
4.	62.5	80.62 ± 2.56
5.	31.25	94.43 ± 2.63
6.	15.625	98.42 ± 2.60

Based on the toxicity test of Mandarin Orange peel ethanol extract, the lowest concentration, which is 15.625 µg/ml, was able to eliminate 1.58% WiDr colon cancer cells, while

the highest concentration (500 µg/ml) was able to eliminate the WiDr colon cancer cells up to 94.88%. This indicated that, the higher the concentration leads to the lower the cell viability. This result was strengthened with the linear graph (Figure 1) of the WiDr cell viability and the concentration of Mandarin Orange peel ethanol extract (IC<sub>50</sub> is 240 µg/ml).

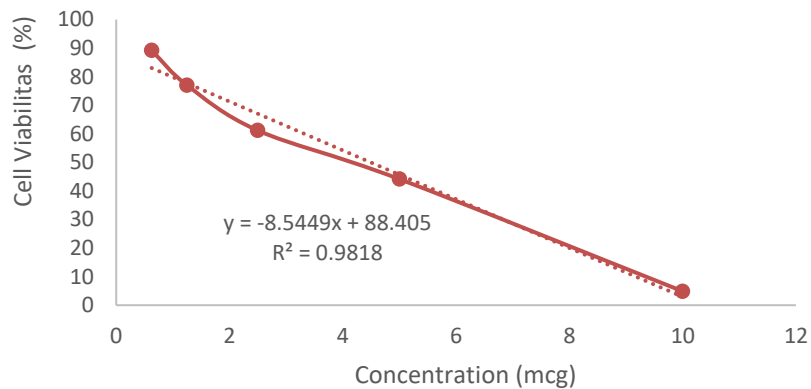
The cytotoxicity test result showed a good positive R<sup>2</sup> value, which is 0.9707, and indicates that the higher the Mandarin Orange peel ethanol extract doses, the lower the WiDr cancer colon cell viability. This result exhibited that the extract has a cytotoxicity effect on WiDr colon cancer cells by a dose-dependent manner. The IC<sub>50</sub> value of Mandarin Orange (*Citrus reticulata*) peel ethanol extract is 240 µl/ml, which is classified as moderate cytotoxicity and could be used as a colon cancer preventive agent. Previous studies have inline results with this study. Mandarin Orange (*Citrus reticulata*) peel ethanol extract with various doses (10 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml, and 250 µg/ml) has 184.5 µg/ml IC<sub>50</sub> value, which is classified as moderate cytotoxicity and could be used as colon cancer medication (Astuti & Primasari, 2020). Another previous study (Wen et al., 2020), showed that Mandarin Orange (*Citrus reticulata*) peel contains flavonoids that could be used for cancer prevention and has low toxicity. Furthermore, flavonoids can resist the activity of Cdk (Cyclin-Dependent Kinase), through the inhibition of the CAK (Cdk-Activating Kinase) enzyme, so the complex of Cdk-Cyclin is nonactive.

Furthermore, as the positive control, the doxorubicin toxicity test showed that the lowest concentration of doxorubicin (0.3123 µg/ml) was able to eliminate 16.55% of WiDr colon cancer cells, and the highest concentration of doxorubicin (10 µg/ml) was able to eliminate 88.65% of WiDr colon cancer cell, as shown in Table 2.



**Figure 1.** The linear equation between WiDr cell viability (%) and Mandarin Orange peel ethanol

extract  
(EEKJM) Dose. The R<sup>2</sup> showed 0.9703



**Figure 2.** Linear relation between WiDr cell viability (%) and Doxorubicin

**Table 2.** Average of colon cancer cell WiDr viability against doxorubicin.

	Doxorubicin Concentration (µg/ml)	Average of Cell Viability (%)
1.	10	11.35 ± 0.82
2.	5	11.93 ± 0.70
3.	2.5	16.05 ± 1.84
4.	1.25	43.97 ± 0.96
5.	0.625	69.08 ± 0.72
6.	0,313	83,45 ±1.18

This indicated that the higher the concentration of doxorubicin, the lower the cell viability. Based on Linear relation as shown in Figure 2, the result of IC<sub>50</sub> of doxorubicin on WiDr Cell is 1.59 µg/ml.

The cytotoxicity test of doxorubicin against WiDr colon cancer cells showed a positive R<sup>2</sup> value, which is 0.5622; and WiDr colon cancer cells have low viability with the highest doses of doxorubicin, as a dependent dose manner pattern. Moreover, The IC<sub>50</sub> value of doxorubicin is 1.59 µg/ml, which is classified as potential cytotoxicity. This result is in line with (Fathani & Miladiyah, 2021), and (Nuryastuti et al., 2017), which have done the doxorubicin toxicity test against WiDr colon cancer cells with IC<sub>50</sub> values of 3.499 µg/ml and 2.295 µg/ml respectively. Doxorubicin itself is a cancer chemotherapy agent with a wide spectrum, well price efficiency, and is used in colon cancer testing (Utami et al., 2020).

Moreover, the VEGF concentration percentage of WiDr colon cancer cells with Mandarin Orange peel ethanol extract and with doxorubicin treatments are shown in Table 3 and

Table 4.

**Table 3.** VEGF concentration percentage of WiDr colon cancer cell with Mandarin Orange (*Citrus reticulata*) ethanol extract treatment.

No.	Sample (µg/ml)	VEGF Level (ng/ml)
1	Control Cell	899.95 ± 9.12
2	120 µg/ml	450.0 ± 26.87
3	60 µg/ml	630.8 ± 11.3

Based on Table 3, the VEGF level of WiDr colon cancer cells was affected by Mandarin Orange (*Citrus reticulata*) ethanol extract. At the doses of 120 µg/ml, the decrease in VEGF level was higher than at the doses of 60 µl/ml. So, the higher doses lead to lower VEGF concentrations of WiDr colon cancer cells.

**Table 4.** VEGF concentration percentage of WiDr colon cancer cell with doxorubicin treatment.

No.	Sample(µg/ml)	VEGF Level(ng/ml)
1.	Control Cell	899.9 ± 9.12
2.	0.795 µg/ml	81.5 ± 3.74
3.	0.3975 µg/ml	77.3 ± 1.48

Based on Table 4, the VEGF level of WiDr colon cancer cells was treated with doxorubicin as a positive control, and it showed that 0.795 µg/ml dose was given a higher decreasing level of VEGF compared with 0.3975 µg/ml dose. So, the higher dose leads to lower VEGF concentration of WiDr colon cancer cells.

In this study, VEGF (Vascular Endothelial

Growth Factor) concentrations are decreased because of Mandarin Orange (*Citrus reticulata*) peel ethanol extract and doxorubicin treatments. This result is based on various groups that have been done: negative control, positive control (doxorubicin), and extract samples (120 µg/ml and 60 µg/ml). Measurement of VEGF concentration using an ELISA reader showed that the highest extract given to WiDr colon cancer cells would generate the lowest VEGF concentration. The VEGF concentration with extract treatments is higher than the VEGF concentration with doxorubicin treatments, this is because doxorubicin is a patent medicine which already used as a cancer treatment. Furthermore, doxorubicin could resist the Topoisomerase II enzyme which leads to DNA damage and apoptosis and directs to a decrease in VEGF concentration level in WiDr colon cancer cells drastically. Whereas the Mandarin Orange (*Citrus reticulata*) peel ethanol extract acts as retardation of angiogenesis through the decrease of VEGF concentration. This is because the flavonoids contained in Mandarin Orange (*Citrus reticulata*) peel could resist cancer cell proliferation and decrease the VEGF expression in ovarian cancer cells. In addition, nobiletin could resist angiogenesis mediator secretion such as Akt, HIF-1 $\alpha$ , and VEGF in ovarian cancer cells (He et al., 2015). Tangeritin affects anti-metastasis activity and anti-angiogenic activity by decreasing the MMPs and VEGF expression in breast cancer (Arivazhagan & Sorimuthu Pillai, 2014), whereas luteolin is one of the flavonoids that could resist angiogenesis in stomach cancer (Zang et al., 2017). Meanwhile, the anti-angiogenic medications' effects have been so unsatisfactory thus far. It is most likely because they have been used in a manner known as "carpet bombing," as opposed to utilizing them by the biology of the tumor (Ribatti et al., 2021).

Current research has found the ability of citrus reticulate skin to reduce VEGF scores as well as cell survival of the WiDr cell line as a model of colon cancer. This potential requires more confirmation in higher experiments with animal models and more exploration in the field of anti-cancer. The main problem with the use of anti-angiogenic drugs to treat tumors is that they are used at the wrong stage of the disease. Given the current stage of tumor growth, several

combinations of treatment can be adjusted in this context. So there are still a lot of opportunities to develop drugs that are targeted against the growth of tumors or cancer cells. The ideal strategy needs to depend on the use of one inhibitor that targets several characteristics of cancer, namely, tumor cell proliferation/stemness, and angiogenesis.

## CONCLUSION

The conclusion of this study is Mandarin Orange (*Citrus reticulata*) peel ethanol extract has 240 µg/ml of IC<sub>50</sub> value against WiDr colon cancer cells, and is classified as moderate cytotoxicity, therefore, Mandarin Orange (*Citrus reticulata*) peel ethanol extract and doxorubicin could decrease the VEGF level in WiDr colon cancer cells. Next research is needed to explore the way of decreasing VEGF by Mandarin oranges also using animal models to explain the effect of mandarin oranges in the cancer field more advance.

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## REFERENCES

- Ademosun, A. O., Oboh, G., Passamonti, S., Tramer, F., Ziberna, L., Boligon, A. A., & Athayde, M. L. (2015). Inhibition of metalloproteinase and proteasome activities in colon cancer cells by citrus peel extracts. *Journal of Basic and Clinical Physiology and Pharmacology*, 26(5), 471–477. <https://doi.org/10.1515/jbcpp-2013-0127>
- Apte, R. S., Chen, D. S., & Ferrara, N. (2019). VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell*, 176(6), 1248–1264. <https://doi.org/10.1016/j.cell.2019.01.021>
- Arivazhagan, L., & Sorimuthu Pillai, S. (2014). Tangeretin, a citrus pentamethoxyflavone, exerts cytostatic effect via p53/p21 up-regulation and suppresses metastasis in 7,12-dimethylbenz( $\alpha$ )anthracene-induced rat mammary carcinoma. *The Journal of Nutritional Biochemistry*, 25(11), 1140–1153. <https://doi.org/10.1016/j.jnutbio.2014.06.007>
- Astuti, Y., Priambodo, W., Harimurti, S., & Suharto, A. (2021). *Apoptosis Effect Of Ethanol Extract*

- oF Saoursoup Leaves on Widr Cancer Cell Line*. International Conference on Sustainable Innovation (ICoSI).
- Astuti, Y., Priambodo, W. J., Rahmah, & Septiyana, D. A. (2021). Overview of wound assay methods extracts ethanol leaves soursop (*Annona muricata*) on wider colon cancer cells. *Bali Medical Journal*, 10(3), 1385–1389. <https://doi.org/10.15562/bmj.v10i3.2979>
- Astuti, Y., & Primasari, A. (2020). Ethanolic Extract of Citrus reticulata Peel Inhibits the Migration of WiDr Colon Cancer Cells. *Indonesian Journal of Cancer Chemoprevention*, 11(2). <https://doi.org/10.14499/indonesianjcanchemoprev11iss2pp60-66>
- Astuti, Y., Suharto, A., Harimurti, S., & Priambodo, W. (2022). The Anti-Proliferative Ability of Ethanol Extract of *Annona muricata* Leaves on WiDr Cancer Cell. *Mutiara Medika: Jurnal Kedokteran Dan Kesehatan*, 22(1), 38–43.
- Celano, M., Maggisano, V., De Rose, R. F., Bulotta, S., Maiuolo, J., Navarra, M., & Russo, D. (2015). Flavonoid Fraction of *Citrus Reticulata* Juice Reduces Proliferation and Migration of Anaplastic Thyroid Carcinoma Cells. *Nutrition and Cancer*, 67(7), 1183–1190. <https://doi.org/10.1080/01635581.2015.1073760>
- De Palma, M., Biziato, D., & Petrova, T. V. (2017). Microenvironmental regulation of tumor angiogenesis. *Nature Reviews Cancer*, 17(8), 457–474. <https://doi.org/10.1038/nrc.2017.51>
- Elebiyo, T. C., Rotimi, D., Evbuomwan, I. O., Maimako, R. F., Iyobhebhe, M., Ojo, O. A., Oluba, O. M., & Adeyemi, O. S. (2022). Reassessing vascular endothelial growth factor (VEGF) in anti-angiogenic cancer therapy. *Cancer Treatment and Research Communications*, 32, 100620. <https://doi.org/10.1016/j.ctarc.2022.100620>
- Fakhri, S., Abbaszadeh, F., Jorjani, M., & Pourgholami, M. H. (2021). The effects of anticancer medicinal herbs on vascular endothelial growth factor based on pharmacological aspects: A review study. *Nutrition and Cancer*, 73(1), 1–15. <https://doi.org/10.1080/01635581.2019.1673451>
- Fathani, I. J., & Miladiyah, I. (2021). Cytotoxicity of ethanolic extract of fruit shells and seeds of Nyamplung (*Calophyllum inophyllum* L.) on WiDr colorectal cancer cells. *Jurnal Kedokteran Dan Kesehatan Indonesia*. <https://doi.org/10.20885/JKKI.Vol12.Iss2.art10>
- Ghalehandi, S., Yuzugulen, J., Pranjol, M. Z. I., & Pourgholami, M. H. (2023). The role of VEGF in cancer-induced angiogenesis and research progress of drugs targeting VEGF. *European Journal of Pharmacology*, 949, 175586. <https://doi.org/10.1016/j.ejphar.2023.175586>
- He, Z., Li, B., Rankin, G. O., Rojanasakul, Y., & Chen, Y. C. (2015). Selecting bioactive phenolic compounds as potential agents to inhibit proliferation and VEGF expression in human ovarian cancer cells. *Oncology Letters*, 9(3), 1444–1450. <https://doi.org/10.3892/ol.2014.2818>
- Henri, H., Sari, D. P., & Hakim, L. (2022). Medicinal Plants for Traditional Treatment Used by the Malays in South Bangka Regency, Indonesia. *Biosaintifika: Journal of Biology & Biology Education*, 14(1), 125–134. <https://doi.org/10.15294/biosaintifika.v14i1.34455>
- Kristiani, E. B. E., & Kasmiyati, S. (2021). The Combination of Phyllanthus niruri, Euphorbia hirta, and Loranthus sp. As a Source of Antioxidant Agents. *Biosaintifika: Journal of Biology & Biology Education*, 13(2), 201–211. <https://doi.org/10.15294/biosaintifika.v13i2.30718>
- Liu, Y., Han, Z., Zhang, S., Jing, Y., Bu, X., Wang, C., Sun, K., Jiang, G., Zhao, X., Li, R., Gao, L., Zhao, Q., Wu, M., & Wei, L. (2011). Effects of Inflammatory Factors on Mesenchymal Stem Cells and Their Role in the Promotion of Tumor Angiogenesis in Colon Cancer. *Journal of Biological Chemistry*, 286(28), 25007–25015. <https://doi.org/10.1074/jbc.M110.213108>
- Liu, Z.-L., Chen, H.-H., Zheng, L.-L., Sun, L.-P., & Shi, L. (2023). Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Signal Transduction and Targeted Therapy*, 8(1), 198. <https://doi.org/10.1038/s41392-023-01460-1>
- Ntellas, P., Mavroeidis, L., Gkoura, S., Gazouli, I., Amylidi, A.-L., Papadaki, A., Zarkavelis, G., Mauri, D., Karpathiou, G., Kolettas, E., Batistatou, A., & Pentheroudakis, G. (2020). Old Player-New Tricks: Non-Angiogenic Effects of the VEGF/VEGFR Pathway in Cancer. *Cancers*, 12(11), 3145. <https://doi.org/10.3390/cancers12113145>
- Nuryastuti, T., Pratiwi, R., & Puniawati Soesilo, N. (2017). 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*, 5(2), 128–137. <https://doi.org/10.24252/bio.v5i2.3944>
- Parveen, A., Subedi, L., Kim, H. W., Khan, Z., Zahra, Z., Farooqi, M. Q., & Kim, S. Y. (2019). Phytochemicals Targeting VEGF and VEGF-Related Multifactors as Anticancer Therapy.

- Journal of Clinical Medicine*, 8(3), 350. <https://doi.org/10.3390/jcm8030350>
- Prastiyanto, M. E., Rukmana, R. M., Saraswati, D. K., Darmawati, S., Maharani, E. T. W., & Tursinawati, Y. (2020). Anticancer potential of methanolic extracts from *Pleurotus* species on raji cells and antibacterial activity against Methicillin-Resistant *Staphylococcus aureus*. *Biodiversitas*, 21(12), 5644–5649. <https://doi.org/10.13057/biodiv/d211221>
- Raza, W., Luqman, S., & Meena, A. (2020). Prospects of tangeretin as a modulator of cancer targets/pathways. *Pharmacological Research*, 161, 105202. <https://doi.org/10.1016/j.phrs.2020.105202>
- Ribatti, D., Solimando, A. G., & Pezzella, F. (2021). The Anti-VEGF(R) Drug Discovery Legacy: Improving Attrition Rates by Breaking the Vicious Cycle of Angiogenesis in Cancer. *Cancers*, 13(14), 3433. <https://doi.org/10.3390/cancers13143433>
- Riihimäki, M., Hemminki, A., Sundquist, J., & Hemminki, K. (2016). Patterns of metastasis in colon and rectal cancer. *Scientific Reports*, 6(1), 29765. <https://doi.org/10.1038/srep29765>
- Rivankar, S. (2014). An overview of doxorubicin formulations in cancer therapy. *Journal of Cancer Research and Therapeutics*, 10(4), 853. <https://doi.org/10.4103/0973-1482.139267>
- Schirmacher, V. (2018). From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review). *International Journal of Oncology*, 54(2), 407–419. <https://doi.org/10.3892/ijo.2018.4661>
- Utami, D. T., Nugraheni, N., Jenie, R. I., & Meiyanto, E. (2020). Co-treatment of Brazilein Enhances Cytotoxicity of Doxorubicin on WiDr Colorectal Cancer Cells Through Cell Cycle Arrest. *The Indonesian Biomedical Journal*, 12(4), 376–383. <https://doi.org/10.18585/inabj.v12i4.1293>
- Van Der Zanden, S. Y., Qiao, X., & Neefjes, J. (2021). New insights into the activities and toxicities of the old anticancer drug doxorubicin. *The FEBS Journal*, 288(21), 6095–6111. <https://doi.org/10.1111/febs.15583>
- Wang, F., Huang, Y., Wu, W., Zhu, C., Zhang, R., Chen, J., & Zeng, J. (2020). Metabolomics Analysis of the Peels of Different Colored Citrus Fruits (*Citrus reticulata* cv. ‘Shatangju’) During the Maturation Period Based on UHPLC-QQQ-MS. *Molecules*, 25(2), 396. <https://doi.org/10.3390/molecules25020396>
- Wen, S., Sun, L., An, R., Zhang, W., Xiang, L., Li, Q., Lai, X., Huo, M., Li, D., & Sun, S. (2020). A combination of *Citrus reticulata* peel and black tea inhibits migration and invasion of liver cancer via PI3K/AKT and MMPs signaling pathway. *Molecular Biology Reports*, 47(1), 507–519. <https://doi.org/10.1007/s11033-019-05157-z>
- Wu, J., Tang, Y., & Liang, X. (2018). Targeting VEGF pathway to normalize the vasculature: An emerging insight in cancer therapy. *OncoTargets and Therapy*, Volume 11, 6901–6909. <https://doi.org/10.2147/OTT.S172042>
- Yang, Y., & Cao, Y. (2022). The impact of VEGF on cancer metastasis and systemic disease. *Seminars in Cancer Biology*, 86, 251–261. <https://doi.org/10.1016/j.semcancer.2022.03.011>
- Zang, M., Hu, L., Zhang, B., Zhu, Z., Li, J., Zhu, Z., Yan, M., & Liu, B. (2017). Luteolin suppresses angiogenesis and vasculogenic mimicry formation through inhibiting Notch1-VEGF signaling in gastric cancer. *Biochemical and Biophysical Research Communications*, 490(3), 913–919. <https://doi.org/10.1016/j.bbrc.2017.06.140>