

Resistivity of Ant Nest (*Myrmecodia pendans*) on Ethanol Fraction Burkitt's Lymphoma Cancer Cells (Invitro) Through Interleukin 8 Angiogenesis Obstacles (II-8)

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Abstract

This study aims to know how the inhibitory power of angiogenesis interleukin 8 ethanol fraction of ant nest extract in Burkitt's lymphoma cancer cell culture.

The study was conducted in a pure laboratory experimental method using Burkitt's lymphoma cancer cell cultures. Gradual research began with determination, extraction, fractionation, making DMEM medium, activation of Burkitt's Lymphoma cancer cells, up to cytotoxicity test and angiogenesis inhibition test of flavonoid fraction extract of ant nests. Data were analyzed using two-way ANOVA followed by Post Hoc LSD (Least Significant Difference) test with a significance level of 95%. Cytotoxicity test results showed that the flavonoid ethyl acetate fraction at a concentration of 1000 µg / mL could cause cell death as much as 82.71%, and at the lowest concentration of 7.8125 µg / mL can cause cell death by 39.75% cells.

The results of the angiogenesis inhibition test showed a significant change in the expression of interleukin 8 in standard solutions. Changes in the expression of interleukin 8 in ethanol solution and ethyl acetate solution with a concentration of 0 to a concentration of 125 results obtained continued to increase. However, when the ethanol solution with 250 levels, the results obtained decreased from 0.254 to 0.237.

Likewise, changes in the expression of interleukin 8 in ethyl acetate solution with a concentration of 250 results obtained decreased from 0.291 to 0.261.

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Introduction

Cancer is a deadly disease and the leading cause of death in industrialized countries and the second cause of death in developing countries. As many as 12.7 new cases of cancer are reported each year with a mortality rate of 7.6 million worldwide¹. World Health Organization (WHO) reports the top five types of cancer found in men in the world in 2012, namely lung cancer,

prostate cancer, colorectum, stomach cancer and liver cancer. Whereas most women are cancers of the breast, colorectum, lung, cervix and stomach cancer².

Cancer is a general term for a large group of diseases that can affect every part of the body. Another term used is malignant tumors and neoplasms. One of the defining features of cancer is the abnormal growth of new cells that grow beyond normal limits, and which can then attack the body's parts and spread to other organs³. The process of occurrence of cancer (oncogenesis) in children is the same as in adults viewed from the biomolecular aspect, the fundamental difference is in the process of the course of the disease. Cancer in children usually occurs at a later stage than in adults when diagnosing it. Cancer in children tends to be

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more aggressive, this is because cancer cells in children are still primitive cells so it is easier and faster to spread. The tendency of cancer to occur in certain places is also a characteristic difference in children^{4,5}.

Lymphoma is defined as a malignant disease that attacks lymphocytes that are in lymphoid tissues such as lymph nodes. This disease was first described by Thomas Hodgkin in 1832 in London England⁶. Lymphoma is the third fastest growing and developing cancer after skin cancer and lung cancer. It is estimated that around 1.5 million people in the world live with non-Hodgkin's lymphoma (LNH), and about 300,000 people a year die of this disease. In Indonesia, lymphoma or more often is called gland cancer which is a group of the top ten malignant cancers⁴.

Lymphoma is divided into two main groups, namely: non-Hodgkin's lymphoma (LNH) and Hodgkin's lymphoma (LH). Burkitt's Lymphoma is a type of LNH cancer with high gradation ability and is formed from small, noncleaved, undifferentiated, diffuse and derived lymphocyte cells. Proliferation and multiplication of Burkitt lymphoma is very aggressive, so patients usually die quickly⁷. Burkitt's lymphoma was discovered by Dr. Dennis Burkitt in 1950. Burkitt's lymphoma has the highest rate of human cell proliferation, cell malignancies from BL have a proliferation index (Ki67 > 95%) and doubling times are 24-26 hours per day and diameters reach 10-15 cm in some cases⁸.

In general, clinical signs and symptoms seen in Burkitt's lymphoma include lesions that occur in the areas of the maxilla, mandible and abdomen. The most commonly seen signs are local tumors in the oral cavity and tooth mobility. As for local pain, tenderness, paresthesia is rarely found. The presence of Burkitt's lymphoma radiographs such as radiolucency due to bone destruction with unclear / diffuse limits. "Starry sky" microscopic appearance, monomorphic, immature, and interspaced undifferentiated lymphocytes are characteristic of Burkitt's lymphoma⁹.

The results showed that ant nest plant is one of the medicinal plants believed to have potential effects in the world of health. Although modern therapies such as chemotherapy give positive results in the treatment of cancer, on the other hand many cause side effects. Therefore, herbal treatment is often a cancer treatment

option. In addition to the low cost, the side effects produced are also minimal compared to modern therapies^{10,11}.

M. Ahkam Subroto, LIPI's main research expert revealed that the active compounds contained in ant nest plants are flavonoids, tannins and polyphenols which function as antioxidants in the body. The active compounds of polyphenols contained in ant nests have many properties, namely as antimicrobial, antidiabetic, and anticancer. Normally every research on natural materials that are suspected as potential drugs or empirically has been used by the community as a drug, starting with pre-clinical toxicity tests to predict their safety level, then followed by other pharmacological tests. Toxicity test methods can be carried out in vitro. Several studies in vitro testing, proved that ant nests are effective in overcoming cancer cells¹². This preclinical test is designed to know the toxic effects of a substance that arises after a single or repeated administration of a substance in 24 hours. Toxic effects in question are all effects that reduce the ability of organ functions to interfere with the organism's functional or biochemical in general¹³.

Interleukin 8 is an oncoprotein from the chemokine family, produced by various cells, including cancer cells. IL-8 is not present in physiological angiogenesis but is found in cancer angiogenesis. The presence of IL-8 together with VEGF is an indicator of the occurrence of angiogenesis. Cytokine protein IL-8 has been shown to play a role in the inflammatory process, tumorigenesis, angiogenesis, through the formation of microvessels in tumors and metastases. IL-8 can stimulate endothelial adhesion, trigger transendothelial migration, and activate neutrophils. In addition, IL-8 can act as a chemotactic factor against T cell infiltration thus it will increase its infiltration in the Naso Faring Chains network. Research into the role of IL-8 has been shown in malignant melanoma to increase its metastatic potential¹⁴.

This study was conducted to know the cytotoxic effect of using ethanol extract of ant nest plants as anticancer in cancer of Burkitt's lymphoma. In this study, the effect of ethanol extract on ant nest plants on the culture of Burkitt's lymphoma cancer cells will be tested.

Materials and methods

Research on the inhibition of cytotoxicity tests on angiogenesis interleukin 8 in the ethanol fraction of ant nest extract (*Myrmecodia Pendans*) in Burkitt's lymphoma cancer cells was carried out in April-June 2018. This was a purely laboratory experimental research using Burkitt's Lymphoma cancer cell cultures conducted in Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University in March-April 2018, and at an integrated research laboratory, Faculty of Dentistry, Gadjah Mada University, Yogyakarta in May-June 2018.

The research raw materials used were to obtain pure compounds namely ant nest plants (*myrmecodya pendans*) obtained and imported from Papua region and then processed in the LPPT laboratory to obtain the fraction of ant nest extracts needed for research..

The procedure of this research is through two stages. The first phase of the study consisted of 7 phases. The first phase is the determination of ant nests. This procedure aims to find out the type of ant nest used and to avoid errors in the plants used in the research test. The second phase is extraction. Extraction was carried out by maceration to obtain ant nest ethyl acetate extract. Ant nests obtained from Papua were cleaned from dirt. Then chopped into small pieces and dried in the open air. Bulbs that have been dried were ground into simplicia powder. The simplicia powder of the ant nest was put into the maserator whose bottom has been coated with cotton. Then into the maserator ethyl acetate - water was added with a ratio of 9: 1. The maceration process was allowed to stand for 24 hours, while stirring occasionally. After 24 hours, the mass was removed and accommodated. All the results of the solvent collection were mixed and then the extraction process was concentrated using a rotary evaporator until all the solvents evaporated and a concentrated ethanol extract was obtained. The next phase was fractionation, the process of compound separation based on the polarity level. Next, activation and breeding of burkitt lymphoma cancer cells were performed. And finally the cytotoxicity test was carried out by incubating the

cell with the amount 2×10^4 of ceels for 24 hours with a series of ant nest concentration flavonoids. In the second phase of the study then carried out angiogenesis inhibition test using interleukin 8 (Il-8) protein and data analysis was done using two-way ANOVA followed by Post Hoc LSD (Least Significant Difference) test with a significance level of 95%. Pearson correlation test was conducted to see a strong relationship between variables. Statistical analysis was carried out using SPSS 16.0 program. Ethical clearance conducted at the Gadjah Mada University Faculty of Dentistry Ethics and Advocacy Unit No.00761/KKEP/FKG-UGM/EC 2016.

Results

The results of the cytotoxicity test in the Table 1 showed that the percentage of Burkitt's lymphoma cancer cell death continued to increase along with the increasing concentration given to each fraction, both ethanol, ethyl acetate, hexan and water fractions. Flavonoids of ethyl acetate and flavonoid fractions of ethanol fraction produced a higher percentage of cell death than the hexan fraction and the water fraction for the highest concentration of $1000 \mu\text{g} / \text{mL}$. While for the lowest concentration was $7.8125 \mu\text{g} / \text{mL}$, the best cell growth resistance was obtained from the flavonoids hexan fraction and water fraction.

Flavonoid ethyl acetate fraction at the highest concentration of $1000 \mu\text{g} / \text{mL}$ resulted in 82.71% of cell death percentage, and the lowest concentration of $7.8125 \mu\text{g} / \text{mL}$ caused cell death of 39.78% cells. For flavonoids, the flavonoid fraction at the highest concentration of $1000 \mu\text{g} / \text{mL}$ resulted in a mortality percentage of 75.37% and konsentrasi terendah sebesar $7,8125 \mu\text{g}/\text{mL}$ menyebabkan kematian sel sebesar 33,18 %. Sedangkan untuk flavanoid fraksi hexan pada konsentrasi $1000 \mu\text{g}/\text{mL}$ menghasilkan persentase kematian sebesar 62,76 % dan konsentrasi terendah $7,8125 \mu\text{g}/\text{mL}$ menyebabkan kematian sel sebesar 31,6%. Finally, the flavanoid fraction of water with a concentration of $1000 \mu\text{g} / \text{mL}$ produced a mortality percentage of 46.1% and the lowest concentration of $7.8125 \mu\text{g} / \text{mL}$ caused cell death of 25.8%.

Level (µg/ml)	Log of Level	% Cell Death				Probit			
		Ethyl acetate	Ethanol	Hexan	Water	Ethyl acetate	Ethanol	Hexan	Water
1000	4.80	82.71	75.37	62.76	46.1	5.38	5.38	5.08	4.9
500	3.980	76.12	55.58	49.18	36.5	5.03	4.90	4.72	4.6
250	3.927	56.51	39.00	48.82	35.6	4.72	4.45	4.59	4.6
125	3.097	51.97	36.48	49.49	34.0	4.56	4.36	4.53	4.6
62.5	2.996	47.87	36.36	36.71	30.2	4.39	4.36	4.45	4.5
31.25	2.195	44.28	34.15	35.22	32.9	4.36	4.29	4.42	4.6
15.625	1.894	40.28	33.57	34.64	29.9	4.36	4.27	4.42	4.5
7.812	1.093	39.75	33.18	31.6	25.8	4.01	4.26	4.33	4.4

Table 1. Cytotoxicity test results of ethyl acetate fraction, ethanol fraction, hexan fraction, and water fraction against lymphoma burkitts cancer cells with direct calculation method

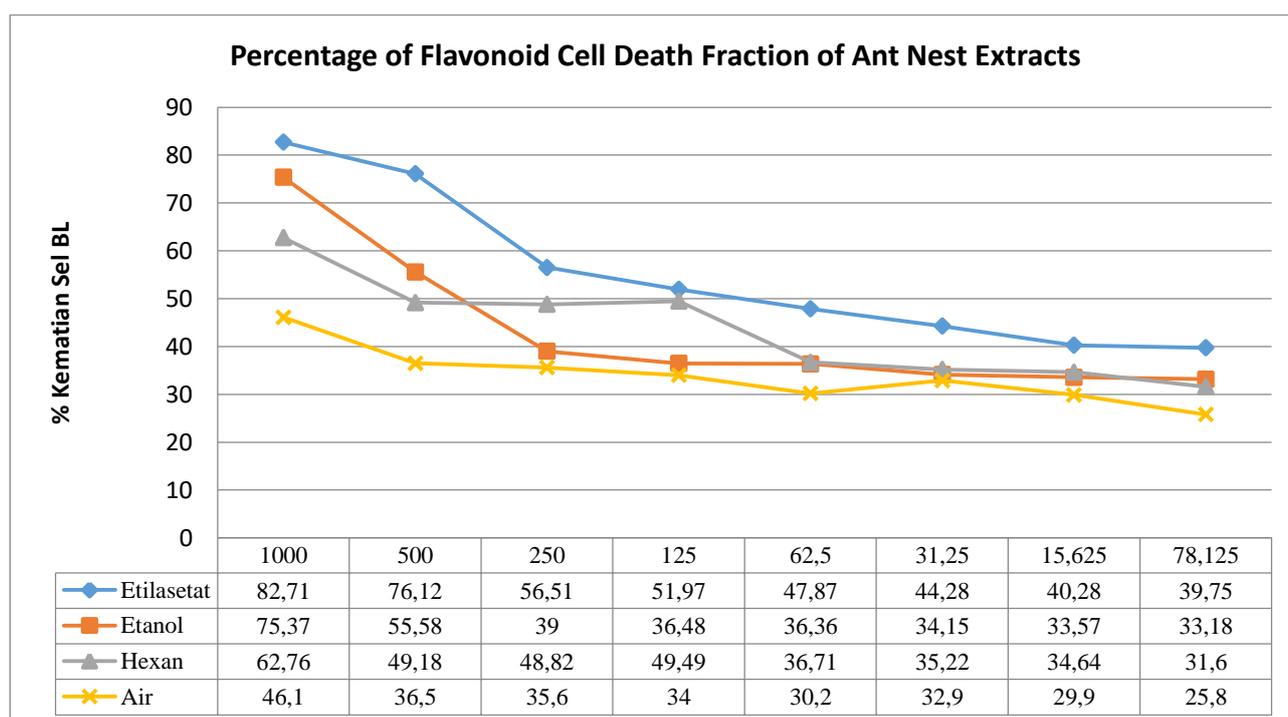


Figure 1. Comparison of the percentage of flavonoid cell death fraction of ant nest extracts.

For comparison of the value of cell growth resistance of each fraction is presented in the diagram above. Where it can be seen for the highest concentration, there was a potential inhibition of cell growth in the flavonoids of ethyl acetate fraction and ethanol fraction. The ethyl acetate fraction obtained 82.71% cell death

levels while the ethanol flavanoid fraction with cell death levels was 75.37%. For the lowest concentration, the best cell growth inhibition value was obtained from flavonoids hexan fraction of 31.6% and water fraction of 25.8% (figure 1).

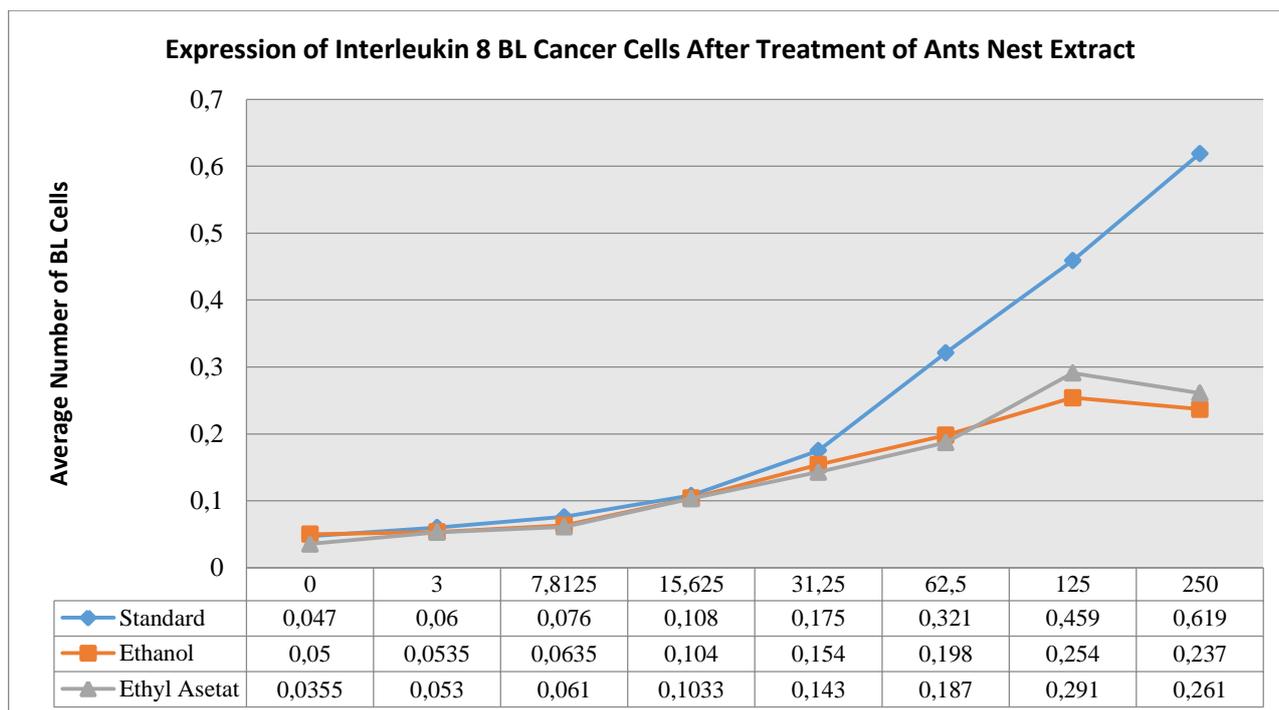


Figure 2. Interleukin 8 expression in post-treatment burkitts lymphoma cancer cells with flavonoid extracts and controls.

Based on figure 2, the results of the research on the inhibition of angiogenesis interleukin 8 showed that there was a significant change in the expression of interleukin 8 in the standard solution. This can be seen when the standard solution with a concentration of 125 results obtained was 0.459. When given a standard solution with a concentration of 250 the results obtained was 0.619. The changes in the expression of interleukin 8 in ethanol solution with a concentration of 0 to a concentration of 125 results obtained continued to increase. However, when the ethanol solution with 250 levels, the results obtained decreased from 0.254 to 0.237. Likewise, the change in the expression of interleukin 8 in ethyl acetate solution with a concentration of 0 to 125 concentrations obtained continued to increase. However, when ethyl acetate solution with a concentration of 250 the results obtained decreased from 0.291 to 0.261.

Discussion

Cytotoxicity tests performed on Burkitt's lymphoma cancer cells showed an increase in the percentage of cell deaths from the four fractions, namely ethanol fraction, ethyl acetate, hexan fraction and water fraction which continued to increase along with the given

concentration increase. Flavonoid fraction of ethyl acetate and ethanol fraction resulted in potential cell death compared to water fraction and hexan fraction. The results obtained from flavonoid ethyl acetate fraction at a concentration of 1000 µg / mL resulted in 82.71% cell death percentage. While the flavonoid ethyl acetate fraction at a concentration of 1000 µg / mL resulted in 75.37% cell death percentage. This proves that ethyl acetate fraction and flavonoid ethanol fraction can provide a significant cytotoxic affected burkitt's lymphoma cancer cells.

From several studies mentioned that ethanol extract from ant nests has toxic abilities from several types of cancer cells. Cytotoxic is the ability of a potential compound to induce cell death, the expected mechanism of cell death is programmed death or apoptosis. Cytotoxic tests were carried out to see the potential of an anticancer drug or the safety of a compound. The system is a qualitative test by determining cell death. The parameter of the cytotoxic test is the IC50 value, which is the value that shows the inhibitory concentration of cell proliferation by 50% and shows the potential toxicity of a compound to the cell. The greater the IC50 value, the more non-toxic. One of the cytotoxic tests that is often used is the MTT test (3-[4,5-dimethylthiazol-2yl] - 2,5-diphenylethrazolium

bromide). This test was based on the conversion of MTT to crystalline formazan by living cells that determine mitochondrial activity. This test is widely used to measure the cytotoxic effects of drugs in vitro on cell lines. MTT reaction is a cellular reduction reaction based on the breakdown of yellow MTT tetrazolium salts into purplish blue formazan crystals¹⁵.

According to Paulina Yessica and Wildan's research, ethanol extract of ant nest plants has cytotoxic effects on MCF-7 cell culture and SiHa cells. When compared with cytotoxic studies of ethanol extract, ant nest plants and IC50 determination of MCF-7 cells (IC50 353,183 µg / ml dose) and SiHa cells (IC50 41.30 µg / ml dose), ethanol extract of ant nest plants has an IC50 dose of 121,059 µg / ml on WiDr cells. This showed that the effectiveness of ethanol extract of ant nest plants was not the same in every cell type. In addition, it should be considered when the drug is consumed by humans, in this case the ethanol extract of the ant nest plant will be strongly influenced by the factors that exist in the human body thus clinical trials were needed before the ethanol extract of ant nest plants can be used as an alternative medicine for colon cancer. The IC50 dose above shows that ethanol extract of ant nest plants has a better cytotoxic effect on WiDr cell culture than MCF-7 cell culture. In the table the percentage of WiDr cell death that uses extracts of ant nest plants and 5-Fluorourasil shows up and down results. This suggests that increasing doses does not always have more cell death effects than lower doses^{16,17}.

According to research by Mardany et al (2016), testing of ethanol extract of ant nest tubers (*Myrmecodia beccarii*) showed an LC50 price of 22.86 ppm, which means that at a concentration of 22.86 ppm the ethanol extract of ant nest tubers (*M. beccarii*) was able to cause death. 50% of *A. salina* Leach shrimp larvae, whereas in the existing research concerning BSLT test on local ant nest (*Myrmecodia* sp.) Ethanol extract showed LC50 price of 61.11 µg / ml (Frengki, 2014), and in BSLT test ethanol extract of ant nest (*M. pendens*) from Papua obtained LC50 of 37.03 µg / ml (Bustanussalam, 2010)¹⁸.

Based on several studies above, it can be concluded that ethanol extract from ant nests has a toxic effect on some cancer cells.

The results of the study of the inhibition of

interleukin 8 angiogenesis showed that there was a significant change in the expression of interleukin 8 in standard solution because it continued to experience an increase in line with the addition of concentration without any obstacles. This can be seen when the standard solution with a concentration of 125 results obtained was 0.459. When given a standard solution with 250 levels of results obtained is 0.619. The changes in the expression of interleukin 8 in ethanol solution with a concentration of 0 to a concentration of 125 results obtained continued to increase the same as the standard solution. However, when the ethanol solution with 250 levels, the results obtained decreased from 0.254 to 0.237. Likewise, the change in the expression of interleukin 8 in ethyl acetate solution with a concentration of 0 to 125 concentrations obtained continued to increase. However, when ethyl acetate solution with a concentration of 250 the results obtained decreased from 0.291 to 0.261. The decrease in ethanol solution and ethyl acetate solution decreased due to the obstacles produced by flavonoid extract from ant nests.

Interleukin-8 is produced by various normal cells and tumor cells. In normal cells, IL-8 acts as a proinflammatory chemokine, whereas in tumor cells IL-8 is widely expressed during angiogenesis. Infiltration of inflammatory cells is activated by cancer which then stimulates the occurrence of angiogenesis. Associated macrophage tumors have been known as candidates for inflammatory cells for tumor angiogenesis. Macrophage which inflates the tumor increases VEGF and TNF-α expression and does not stimulate angiogenesis directly, but by modulating IL-8 induction with a regulated pathway through paracrine and or autocrine mechanisms. Cytokine protein IL-8 has been shown to play a role in the inflammatory process, tumorigenesis, angiogenesis, through the formation of micro vessels in tumors and metastases. IL-8 can stimulate endothelial adhesion, trigger trans endothelial migration, and activate neutrophils. In addition, IL-8 can act as a chemotactic factor on T cell infiltration thus it will increase its infiltration in the NPC network. Research on the role of IL-8 has been shown in malignant melanoma to increase its metastatic potential^{19,20}.

According to Eka Savitri's research (2014), the results obtained can be made as a temporary

conclusion that there is a tendency to increase IL-8 levels in progressive cancers, although it is not statistically significant. Among inflammatory mediators, several cytokines and chemokines, such as tumor necrosis factor (TNF), Interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8) are cytokines that play a role in tumorigenesis. In the inflammation-like microenvironment (in the area around the tumor), interactions between cytotoxic T cells (CTL) and tumor cells are important in the growth of Nasal Faring Cancer (NPC). These interactions can be tumoral T cells (CTL) with a poor prognosis in NPC, this study supports the notion that T cell inflations affects the progression of NPC. This interaction can be mediated by several chemokines or cytokines. Another way of interaction can involve cell contact through the ligand-receptor binding, for example tumor-infiltrating T cells can provide survival signals to KNF cells via CD40-CD40 ligand interaction, preventing tumor cells from CD95-triggered apoptosis. The conclusion of the results of this study, IL-8 is a marker of nasopharyngeal carcinoma and disease progression and the ratio of IL-8 and IL-10 can be used to assess the prognosis of NPC. The results of the IL-8 ratio: IL-10 > 1 indicates a poor prognosis^{10,20}

Conclusions

Flavonoid extracts from ant nest plants have a cytotoxicity effect on Burkitt's Lymphoma cancer cells. The results of the cytotoxicity test study showed an increase in burkitt's cancer cell lymphoma from each treatment increased with increasing concentration. Flavonoid fraction of ethyl acetate and ethanol fraction resulted in potential cell death compared to water fraction and hexan fraction. The results obtained from flavonoid ethyl acetate fraction at a concentration of 1000 µg / mL resulted in 82.71% cell death percentage. While the flavonoid ethyl acetate fraction at a concentration of 1000 µg / mL resulted in 75.37% cell death percentage. The results of the research on the inhibition of angiogenesis interleukin 8 showed that there was a significant change in the expression of interleukin 8 in standard solutions without any obstacles. This can be seen when the standard solution with a concentration of 125 results obtained was 0.459. When given a standard solution with a concentration of 250 the results obtained were 0.619.

Declaration of Interest

None declared.

References

1. Wijaya C. A.. Muchtaridi. Cancer Treatment Through Therapeutic Gen Method. Farmaka. 2017; 15(1): 53.
2. Dewi M. Distribution of Cancer in Indonesia. Basic Health Research 2007. Indonesian Journal Of Cancer. 2017; 11(1): 1-2.
3. Mambodiyanto. Maharani P. Effects of Alternative Medicine as Factors Causing Delay in Medical Treatment of Patients with Breast Cancer in Lumir Health Center. Banyumas Regency. MEDICAL. 2016; 14(3): 1.
4. Dona R.. Sulistyani N.. Nurani L. H. Cytotoxicity and Antiproliferative Test of Ethanol Extract of Leunca Leaves (*Solanum Nigrum*. L.) Against Raji Cells. Yogyakarta. Pharmacia. 2016; 6 (2): 181-90.
5. Achmad H. et. al. Potentially of Extracted Papua's Anthill (*Myrmecodia pendans*) As Antitumor to Emphasis the Expression of Vascular Endothelial Growth Factor Cell Burkitt's Lymphoma Cancer. Asian Jr. of Microbiol. Biotech. Env. Sc. 2018; 20(1): 2018: 108-12
6. Paramartha K.A.. Rena R.A. Characteristics of Malignant Lymphoma Patients in Sanglah Hospital on 2015. E- Jurnal Medika. 2017; 6 (2): 3.
7. God J.. Azizul H. Burkitt Lymphoma: Patogenesis And Immune Evasion. Journal Of Oncology. Charleston. USA. 2010; 10(1): 1-3.
8. Lacasce C. How I Treat Burkitt Lymphoma In Adults. Blood Journal. 2016; 124(19): 2913-6.
9. Musti K. Burrkitt Lymphoma. Journal of Medicine and Health. 2015; 2(2): 501-6.
10. Hajder et al. Biological and Clinical Features of Non Hodgkin Lymphoma in the Elderly. J BUON. 2012; 7(4): 1-8.
11. Achmad H. et al. Apoptosis Induction (Caspase-3. -9) and Human Tongue Squamous Cell Carcinoma VEGF Angiogenesis Inhibition using Flavonoid's Ethyl Acetate Fraction of Papua Ant Hill (*Myrmecodia pendans*). Journal of International Dental and Medical Research. 2018; 10 (1): 276-84.
12. Frengki. Roslizawaty. Pertiwi D. Toxicity Test of Aceh's Local Ants Nest Ethanol Extract (*Myrmecodia* Sp.) With the Bslt Method of Shrimp Larvae. Journal of Medika Veterinaria. 2014; 8(1): 60-1.
13. Hindrasyah D. M.. et al. Acute Toxicity Test of Ethanol Extract of Ant Nest (*Myrmecodia Pendans*) Kalimantan in Swiss Mice (*Mus Musculus*). Pharmamedics Health Magazine. 2011; 3 (1): 189.
14. Savitri E.. Haryana B. C. The Relationship between Il-8 and Il-10 Levels that Influenced the Progression of Nasopharyngeal Carcinoma. Orli. 2014; 44 (1): 45.
15. Kusuma M. A.. Susanti. Akbariani G. Cytotoxic Potential Ethanol Extract of Beruk Leaves (*Crescentia Cujete* L.) to Cancer Cells. 2014; 2(2): 192.
16. Wandy M.. Ratnawati H.. Herdiman H. Cytotoxic Effects of Ethanol Extract of Ants Nest Plant (*Myrmecodia Pendans* Merr & Perry) on Colon Carcinoma in Widr Cell Culture. 2013; 5(1): 150.
17. Achmad H. Akt Signal Transduction Pathways and Nuclear Factor-Kappa B(NF-kB) Transcription as a Molecular Target of Oral Tongue Squamous Cell Carcinoma (SP-C1) Using Papua's Anthill Plant (*Myrmecodia Pendans*). Pakistan Journal of Biological Science. 2018; 19(8): 323-30.
18. Mardany M. P.. et al. Phytochemical Screening and Cytotoxic Activity Test of Semut Sarang Plants (*Myrmecodia Beccarii* Hook.F.) from Merauke Regency. Papua Biology Journal. 2016; 8 (1): 13-22.

19. Kumar. Abbas. Fausto. Phatologic Basis Of Disease. 9th Ed. Philadephia. Elsevier & Saunders. 2014; 1: 459.
20. Bashkim I., Bokonjic D. Short-term low-level laser therapy attenuates inflammation and production of interleukin-1 but elevates the level of matrix metalloproteinase 9 in chronic periodontitis. Journal of international dental and medical research. 2014; 7(1): 7-13
21. Sri L. C., Masulili. Yulianti K., Soedarsono N., Widyastuti Y., Harsas N.A., Maharani D. A. The relationship of academic stress to periodontal status and level of cortisol hormone, interleukin 1- β and interleukin-6 in gingival crevicular fluid (study on profession and specialist dental students faculty of dentistry universitas indonesia, jakarta). Journal of international dental and medical research. 2016; 9(2): 113-7.
22. Wicaksono A., Sri L. C., Masulili, Sulijaya B., Kemal Y., Auerkari E. I. Analysis of Interleukin-1 α Level In The Severity Of Chronic Periodontitis Influenced By Smoking Habit. Journal Of International Dental And Medical Research; 2016; 10: 429-33
23. Putri F. A., Midoen Y. H., Djamal N., Soedarsono N., Soehartono W., Auerkari E. I. Distribution Of Interleukin-6 Gene Polymorphism (-174 G/C) In Head And Neck Cancer Patients In An Indonesian Population: A Preliminary Study. Journal Of International Dental And Medical Research; 2017: 10: 551-4.