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CYTOTOXICITY ASSAY OF WATER, ETHANOL N-HEXANE EXTRACT OF DATES FRUIT (PHOENIX DACTYLIFERA) AGAINST MURINE LEUKEMIA CANCER CELL P388

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Abstract

The World Health Organization (WHO) states, in 2015, there are an estimated 9 million people who die of cancer and in 2030 there are an estimated 11.4 million deaths from cancer. Blood cancer reaches 3% of all cancers found in humans and is most common in children. The cancer drug that is available today has quite severe side effects, therefore in this study an antileukemia activity test will be carried out on water, 70% ethanol, and n-hexane extract of dates (*Phoenix dactylifera*). This study, the extracts from dates will be tested for cytotoxicity on Murine Leukemia P388 cells using the MTT Assay method. The IC_{50} value of extract of dates is used as an assessment of the activity of palm fruit extract as antileukemia. Citotoxicity assay against Murine Leukemia P388 cells by MTT assay, showed IC_{50} value is more than 100 µg/mL for all extracts. This result informed that the water, ethanol, and n-hexane extract of dates fruit are less potencial as a antileukemia agent.

Keywords: Dates fruit, water, ethanol, n-hexane, MTT assay, Anticancer, P388.

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Introduction

The World Health Organization (WHO) revealed that there is an increase in the number of cancer sufferers every year reach 6.25 million people and two-thirds of them come from developing countries, including Indonesia (Ayuni, Rahman, & Ramaita, 2020). In Indonesia, currently it is estimated that there are new cancer sufferers, 1: 1,000 population per year. According to the data, cancer is the fifth cause of death in Indonesia and has increased significantly (Dewi & Hendrati, 2015).

Cancer is the second leading cause of death worldwide after cardiovascular diseases, according to WHO(Sulistiowati, Lolong, & Pangaribuan, 2016). Indeed, lung, breast, stomach, liver and colorectal cancers are the largest causes of death worldwide each year, and therefore the discovery and the development of suitable agents to treat various types of cancer are highly desirable (Kamilah, 2017). Natural products have become a leading category of compounds in improving the rational drug design for novel anti-cancer therapeutics. Blood cancer or what is known as leukemia is a cancer

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that is often found after cervical cancer, liver cancer, breast cancer, and lung cancer (Sudewo, 2012). This disease accounts for 3% of all cancers found in humans and is most often found in children (Saputra, 2021). Leukemia is a malignant disease of the hematopoietic tissue characterized by the replacement of normal bone marrow elements by abnormal blood cells or leukemic cells (Rofinda, 2012). This is caused by the uncontrolled proliferation of blood cell clones. Bleeding is the main cause of morbidity and mortality in acute leukemia. Bleeding complications result in a mortality of 7 - 10% in acute leukemia patients that occur in the first few days or weeks after diagnosis (Yulianti & Adnan, 2020).

Based on hospital statistics in the Hospital Information System (SIRS) in 2006, leukemia cases (5.93%) were ranked fifth after breast cancer, cervical cancer, liver cancer and intra-hepatic bile duct, non-Hodgkin lymphoma from all cancer patients hospitalized in Indonesia (Ma'unah, 2016). The Indonesian Child Oncology Foundation stated that every year 650 new cancer cases were found throughout Indonesia, 150 of which were in Jakarta, and as many as 70% were leukemia or blood cancer sufferers. Generally, children with blood cancer come after entering an advanced stage which is difficult to cure(Lembang, 2011).

One of the causes of cancer is the presence of free radicals. A free radical is an atom that has one or more unpaired electrons in its outer orbit. This is what causes free radicals to be reactive to get their electron pairs. A certain amount of free radicals is needed by the body for physiological processes by means of electron transfer, but if free radicals are present in excessive amounts, there will be oxidative stress where there is an imbalance between the number of free radicals and intra-cell antioxidants.

Free radicals can be neutralized by a variety of spices, fruits and vegetables that contain lots of antioxidants. Antioxidants are compounds that can neutralize free radicals by giving electrons to free radical molecules without disturbing their function at all.

Dates (Phoenix dactylifera) are one of the fruits that have antioxidant effects. Dates contain active alkaloid compounds, flavonoids, steroids, tannins, estertepen, carbohydrates, vitamins, phenolic acids, β-carotene11,13, sugar, protein, fat, 3 fiber, potassium, calcium, iron, chlorine, copper, magnesium, sulfur, phosphorus, and some enzymes (Dewi, 2014). The content of flavonoids, total phenolic, vitamins and β-carotene have antioxidant activity by binding free radicals.

Therefore, in this study, the antileukemia activity of water extract, 70% ethanol, and n-hexane from dates fruit will be tested, which is expected to find new anticancer drugs, especially antileukemia, which are safer for consumption because of dates does not have any side effects, unlike current anticancer drugs.

Research Methods

1. Materials

   Ajwah dates, water, 70% ethanol, and n-hexane, whatsman paper no.41. The materials used in the cytotoxicity test include: test sample, cell culture of Murine Leukemia P-388, DMSO (Dimethyl Sulfoxide), RPMI 1640 media, FBS (Fetal Bovine Serum), PBS (Phosporic buffer solution pH 7.30-7.65), MTT [3- (4,5-dimethyl thiazol-2-il) -2,5-diphenyl tetrazolium bromide], Artonin E, and SDS (Sodium dodecyl Sulfate).

2. Instrumentation

Beaker glass, Erlenmeyer flask, porcelain cup, spatula, analytical scale, a set of vacuum rotary evaporator, oven To test the anticancer activity against the cancer cells Murin leukemia P-388, among others: micro pipette, 96 well microplate, microplate mixer, ELISA Microplate reader, incubator CO_2.

3. Procedure

1). Extract water, ethanol, and n-hexane of dates fruit

Dried powder of dates fruit is Macerated with water, ethanol, and n-heksan as the solvent, the flesh of the dates has been smooth for 3x24 hours, until the residue turns clear (residual and filtrate phases), stirring occasionally. The ratio between each solvent and powder is 1: 2. g. The resulting macerated filtrate was then filtered using Whatsman filter paper no. 41, until the residue is not found. The filtrate obtained is concentrated using a rotary evaporator at 60^{o}C for 5 hours until a thick extract is obtained. Using a temperature of 60^{o}C so that the content contained in the date palm pulp is not lost.

2). Cytotoxicity assay

P388 cells seeded into 96-well plates at an initial cell density of approximately $3x10^4$ cells cm^{-3}. After 24 h of cell transplantation, various concentrations of compound were added. First, the compound was dissolved in DMSO at the required concentration. Subsequent six desirable concentrations of samples were prepared using PBS (phosphoric buffer solution, pH 7.30-7.65). Control wells received only DMSO. The assay was terminated after 48 h incubation period by adding MTT reagent [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for 4h, After a further 4 hours of incubation, 100 ml of 10% SDS-0.01 N HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. Optical density was read by using a micproplate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested contraction of compounds (μM). The IC_{50} value was the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate.

Result and Discussion

The extract yield obtained for each solvent was 2.06% for the n-hexane extract, 22.29% for the ethanol extract, and 28.60% for the water extract. This results show that water is the best solvent in extracting, while n-hexane has the smallest yield as a solvent. Dates contains a lot of sucrose which is hydrophilic, and so does water, therefore water has the biggest yield than others.

Cytotoxicity test of water, ethanol and n-hexane extracts from dates was carried out in vitro against cancer cells of Murine Leukemia P388. Data from the cytotoxicity test results against murine leukemia P388 cancer cells obtained data draw in the form of optical density values. The sample was made into 7 concentration series: 100 ppm, 30 ppm, 10 ppm, 3 ppm, 1 ppm, 0.1 ppm and 0.3 ppm. Then put it in the microplate 96.

then incubated for 48 hours and read with an ELISA reader so that the optical density value was obtained.

After obtaining the optical density value, a curve is made, the sample concentration series is plotted as the x-axis, and the optical density data as the y-axis. From the results of the curve, then the IC50 value is determined. If the extract concentration is below 100 ppm shows cytotoxicity against murine leukemia P388 cancer cells, then the extract has the potential to be a leukemia anticancer. According to Meiyanto, a compound has potential as a chemopreventive compound if it has an IC_{50} value of less than 100 ppm. Artonin E was used aspositive control (IC_{50} 0,3 ppm). From the results of the cytotoxicity test of water, ethanol and n-hexane extracts from dates against Murine leukemia P388 cancer cells, the IC_{50} value were more than 100 ppm. From these results it can be said that the water, ethanol, and n-hexane extracts of dates were less potential as antileukemic agents.

Conclusion

From the results of the extraction process using water, ethanol, and n-hexane as a solvent, the% yield values were 28.60%, 22.60%, and 2.06%, respectively. It can be concluded that water is the solvent that attracts the most solutes in the extraction process.

From the results of the cytotoxicity test on Murin Leukemia cancer cells, the IC50 value for water, ethanol, and n-hexane extracts was greater than 100 ppm. It can be said that water, ethanol and n-hexane extracts are less potential as leukemia anticancer.

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