Development Of Chemotherapeutic In Mangrove Leaves

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Abstract: The 2014 WHO data estimated 15,985 cases of colon cancer patients, 11,787 women in cases. The highest incidence of colorectal cancer in the country, the death rate of men in Indonesia due to colon cancer is 10.2% with 103,100 deaths, while women are 8.5% with 92,000 deaths. The use of conventional chemotherapeutic agents can cause side effects such as cardiotoxic, nausea, diarrhea, and the immune system. Therefore, it is important to look for potential anticancer alternatives including natural sources. One of the natural ingredients that has the potential to be developed as a chemotherapeutic agent is mangrove leaves. In this study, mangrove leaves from three species were extracted by maceration method using n-hexane solvent. Cytotoxic activity of n-hexane extract from three mangrove leaf species (EnHDM) was determined by linking this extract activity to WiDr cells using the MTT method EnHDM which has an IC_{50} value for WiDr cells. The purpose of the extract test was to obtain the smallest IC50 µg / mL, Avicennia alba (EnHDAA) which was 258.14 µg / mL and Avicennia marina (EnHDAM) that is 295.25 µg / mL Therefore, based on the flow and research method, the three extracts were used as test samples for further anticancer testing. Cytotoxic effects are shown by absorbance values, th lue for later use as a material for selectivity index testing (IS) and further testing of anticancer activity. Then in this study, the test was carried out for 24 hours then the smallest IC_{50} value was obtained from n-hexane extract of mangrove leaves of Avicennia lanata (EnHDAL), 243.32 ug/ml. The result showed that Avicennia lanata, Avicennia alba and Avicennia marina had cytotoxic effects on WiDr cells respectively 234.32 ug/ml, 258 ug/ml and 295,25 ug/ml this proved that it had the potential as a compound of anticancer.

Index Terms: concentration, DNA, isolation, NanoVue, nucleic acids, RNA.

1 INTRODUCTION

Cancer is not a contagious disease, but it becomes a serious health problem in any part of the world including in Indonesia [1]. This behavior change occurs because cells express various abnormal proteins. This can occur because the cells concerned mutate, especially from genes that encode proteins that play a role in the cell division cycle. Mutations of some of these genes occur due to the induction of a mutagen. Examples of mutagens include chemicals, radiation, free radicals, and infections from several types of viruses (oncovirus group) [2]. Chemotherapy agents used today generally suppress the growth or proliferation of cancer cells while at the same time causing toxicity to the body because it also inhibits the division of normal cells which proliferate rapidly, including bone marrow, gastrointestinal mucosa, hair follicles and lymphocyte tissue. This raises concerns about the various side effects caused by the use of conventional chemotherapy agents, such as heart (cardiotoxic) disorders, nausea, diarrhea and suppression of the immune system and the occurrence of resistance, thereby increasing public interest in the use of traditional medicines [3].

Potential natural materials developed as chemotherapy agents include mangrove leaves. Mangroves are defined as plant formations of litoral regions that are typical on protected tropical and sub-tropical coasts [4]. Polyisoprenoids are secondary metabolites that are found in mangroves, distributed as dolichol and polyprenol in mangrove leaves and roots. The distribution and diversity of polyisoprenoid compounds in the leaves and roots of 14 species of North Sumatra mangroves and 10 Okinawa mangroves have been analyzed using two-dimensional thin-layer chromatography (2D-TLC), electrospray ionization mass spectrometry (ESI) and high-ESCR methods. performance liquid chromatography (HPLC) [5]. The diversity and distribution of polyisoprenoids in mangroves can be a potential source of compounds for natural medicine, which can open up other possibilities for the use of non-timber forest products from mangrove forests. So far the research that reports the pharmacological activity of polyisoprenoid from mangrove species is still small, so it becomes important to reach the prospects, potential and mechanism of polyisoprenoid in mangroves as pharmaceutical and medicinal natural ingredients [6]. Studies conducted by [17] prove that the methanol extract of Avicennia alba (bark and leaves) has anti-proliferative activity on MCF-7 and T47D cells. In addition, this extract also shows the existence of cytotoxic effects on various cancer cells including HT-29 colon cancer cells (Akter, et al., 2009). Based on previous studies, polyisoprenoids are able to induce inhibition of the cancer cell cycle of colon adenocarcinomas (COLO 320 HSR cells, WiDr cells and LS174 cells) in the G2 / M phase [8] [9]. Polyisoprenoids have also been shown to inhibit the HL-60 leukemia cell cycle in the G1 phase [10] [11]and increase apoptosis in MCF-7 cells [12]. Polyisoprenoid as a chemopreventive agent in colon cancer. Polyisoprenoids from Avicennia marina and Avicennia lanata leaves have colonic anticancer activity [13]. The polyisoprenoid from Avicennia marina has an IS value of 5.195 (> 3) to be classified as highly selective and Avicennia alba has a polyisoprenoid content inducing cell cycle, apoptosis and Cox-2 gene expression in WiDr colon cancer cells. This extract has a mechanism of cell cycle inhibition in the G0-G1 stage, and apoptotic analysis occurs in the early phase of apoptosis in WiDr cells [14].

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2. MATERIAL AND METHODS

2.1 Plant and Chemical Materials

The mangrove species were collected from Lubuk Kertang, Langkat mangrove forest, North Sumatra provence, Indonesia: Avicennia alba, Avicennia lanata and Avicennia marina

2.2 Manufacturing of n-hexane extracts of Mangrove Leaves

Manufacturing of n-hexane extracts of Mangrove Leaves 500 g of simplicia powder from each of the three mangrove leaf species Avicennia alba, Avicennia lanata and Avicennia marina were macerated with a mixture of chloroform: methanol (2: 1, v / v) for 48 hours. Lipid extract from leaves was saponified at 65ºC for 24 hours in 86% ethanol containing 2 M KOH. The unsammonated part was then dissolved with n-hexane, then the solvent was evaporated. Then it is dissolved again in n-hexane, pour or strain. Concentration was carried out using a rotary evaporator at 40ºC. Then dried until a thick extract is obtained [15] [16].

2.3 Isolation WiDr Cells

Cell lines and cell culture conditions WiDr cells, isolated human colon cancer cells from the large intestine of 78-year-old women obtained from the Laboratory of Parasitology collection, Faculty of Medicine, Gadjah Mada University (UGM) (Yogyakarta, Indonesia). WiDr cell lines cultured in RPMI 1640 medium, and supplement with 10% (v/v) fetal bovine serum (FBS), 1% penicillin and streptomycin, fungizone 0.5%, and in a 37°C incubator with 5% CO2 [17].

2.4 Cytotoxic Test

Cytotoxic testing conducted on WiDr colon cancer cells in this study used the MTT method. WiDr colon cancer cells were planted on 96 microplate wells to obtain a density of 1×10^4 cells / wells and incubated in a 5% CO_2 incubator at 37^0C for 24 hours to obtain good growth. After that the medium was replaced with a new one and then extracted test samples from each concentration series (5-Fu was used as a positive control) and incubated at 37^0C in a 5% CO_2 incubator at 37^0C for 24 hours. At the end of the incubation, culture media and samples were removed and cells were washed with PBS. To each well, 100 µL of culture media (RPMI) and 10 µL MTT were added with a concentration of 5 mg / mL. The cells were re-incubated for 3-6 hours in a 5% CO_2 incubator at 37^0C. The reaction was stopped with a 10% SDS reagent in a 0.01 N HCl solution. Then the 96 well dish was wrapped so that it was not translucent and left overnight at room temperature. Absorption was measured by ELISA Reader at a wavelength of 595 nm [18].

2.5 Calculation of percent of cells viability

Based on absorbance data obtained from cytotoxic tests, cells are converted into percent live cells. Percent of living cell is calculated using the formula: % Viability (Live Cell) = (Abs Treatment of Cell-Abs Control Media) / (Abs Control of Media-Abs Control Media) × 100%Cytotoxic activity is expressed by IC50, namely: concentrations that cause death of 50% of the cell population analyzed using SPSS 23 probit analysis with a significance of 0.05 [17].

2.6 Data analysis

IC_{50} cytotoxic test data using Probit analysis test using SPSS. Gene expression data were analyzed using Statistical for social sciences (SPSS) version 22.0. Data is presented as mean ± Standard error means (SEM) and is significant using Duncan test analysis.

3. Result and Disscusion

Cytotoxic test is a preliminary parameter to find out the potential for toxicity of a test material, especially cancer cells, expressed by the IC_{50} parameter. However, cytotoxic tests can also be done to assess the toxicity of a test material to normal cells, so that their effectiveness against cancer cells can be known. Because a good anticancer compound must have the activity of killing or inhibiting cancer cells without disrupting normal cell function. In this study, the cytotoxic test material was n-hexane extract of mangrove leaves (EnHDM) for 48 hours originating from three species of mangrove against colon cancer cells (WiDr) with a concentration series of 1000 µg / mL, 500 µg / mL; 250 µg / mL; 125 µg / mL and 62.5 µg / mL be seen in Table 1.

Table 1 IC_{50} *48 hrs species Avicennia alba, Avicennia lanata and Avicennia marina*

Mangrove species	WiDr cell µg / mL
Avicennia lanata	243,32
Avicennia alba	258,32
Avicennia marina	295,25
5-Fu	17,43

The purpose of the extract test was to obtain the smallest IC50 µg / mL, Avicennia alba (EnHDAA) which was 258.14 µg / mL and Avicennia marina (EnHDAM) that is 295.25 µg / mL Therefore, based on the flow and research method, the three extracts were used as test samples for further anticancer testing. Cytotoxic effects are shown by absorbance values, th lue for later use as a material for selectivity index testing (IS) and further testing of anticancer activity. Then in this study, the test was carried out for 48 hours then the smallest IC_{50} value was obtained from n-hexane extract of mangrove leaves of Avicennia lanata (EnHDAL), 243.32 en analyzed using probit analysis in the SPSS 23 program, then obtained IC_{50} test samples as shown in figure 1.

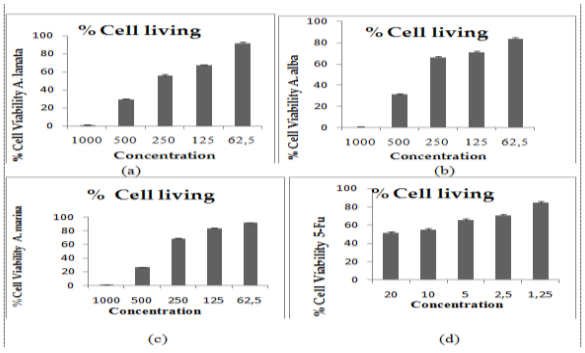

Fig. 1. *Viability cell Cytotoxic effects of test samples on WiDr cell viability. WiDr cells were planted in 96-well plates, then treated with test samples (a) Avicennia lanata (b) Avicennia alba (c) Avicennia marina (d) 5-Fu*

Cytotoxic activity of WiDr cells showed the smallest IC50 value in the treatment with EnHDAL which was 243.32 µg / mL. Therefore, EnHDAL can be said to have the most active anticancer activity because the smallest IC_{50} value is able to inhibit 50% of WiDr cell growth. Based on these values EnHDAL is less active as an anticancer because an extract is considered active if it has an IC_{50} value ≤ 100 µg / mL [19]. However, an extract with an IC_{50} value of 100 - 500 µg / mL

can still be developed as an anticancer with moderate classification [20]. because an extract is considered inactive if the IC_{50} value> 500 µg / mL [21]. Previously, the determination of chemical compounds for n-hexane extracts has been carried out and the result is that there are groups of chemical compounds suspected to be anticancer, namely triterpenoids / steroids. According to [22], triterpenoids / steroids have activities to overcome inflammation, cell proliferation, apoptosis, invasion, metastasis and angiogenesis. Because many of these compounds show good potential in dealing with cancer by various mechanisms, such as regulating the regulation of transcription factors (example: nuclear factor-kappaB [NF-κB], antiapoptotic protein (examples: bcl-2, bcl-xL), triggers from cell proliferation metalloproteinases [MMPs], intracellular adhesion molecules-1 (ICAM-1) and angiogenic protein (vascular endothelial growth factor (VEGF).

4. CONCLUSION

The result showed that Avicennia lanata, Avicennia alba and Avicennia marina had cytotoxic effects on WiDr cells respectively 234.32 ug/ml, 258 ug/ml and 295,25 ug/ml this proved that it had the potential as a compound of anticancer.

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