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CYTOTOXICITY ACTIVITY OF ETHANOL EXTRACT OF MENTANGOR STEM SKIN (*Calophyllum rufigemmatum*) AGAINST BREAST CANCER CELLS MCF-7 IN VITRO

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Abstract: Cancer is one of the non-communicable diseases that causes the most death in Indonesia. In a study conducted by researchers from Northwestern University on 686 breast cancer patients, 36% of women chose to stop their treatment because they could not tolerate the side effects of the drug. The patient's side effects were joint pain, hot flashes, increased weight gain, and nausea. Mentangor plant (*Calophyllum rufigemmatum*) is a plant that grows a lot in Bangka Belitung and has been used by the people of Bangka Belitung as a traditional medicine to treat skin diseases. Research on the bark of the mentangor stem from Bangka Belitung has never been carried out regarding its phytochemical content and potential as an anticancer breast. For this reason, it is necessary to know breast cancer's pharmacological effects and phytochemicals on Mentangor Bangka Belitung's stem bark. Phytochemical identification was carried out qualitatively by observing changes in the test sample. Based on the results of phytochemical screening, several reactions showed that each test of the ethanol extract of the stem bark of Mentangor contained tannins, phenolics, flavonoids, and alkaloids. The measurement results of the IC50 value of inhibition of the proliferation ethanol extract with a diameter of 1 cm, 7 cm, and 17 cm against MCF-7 breast cancer cells were 326,195 g/mL, respectively; 186.178 g/mL; and 178.090 g/mL.

Keywords: *Calophyllum rufigemmatum, Breast Cancer, Phytochemicals, Toxicity*.

INTRODUCTION

Cancer is one of the non-communicable diseases that causes the most death in Indonesia. According to the Global Cancer Observatory Data (2018) from the World Health Organization (WHO), the most cancer cases in Indonesia are breast cancer, reaching 58,256 cases. The Ministry of Health of the Republic of Indonesia (KEMENKES) states that the average death rate from breast cancer reaches 17 people per 100,000 population [1]. The risk factors for breast cancer are obesity, family history, age of giving birth to their first child, history of breastfeeding, age of menarche, age of menopause, and use of contraceptive pills [2]. In a study conducted by researchers from Northwestern University on 686 breast cancer patients, 36% of women chose to stop their treatment because they could not tolerate the side effects of the drug. The patient's side effects were joint pain, hot flashes, increased weight gain, and nausea [3]. Therefore, herbal medicines are needed that do not have side effects on breast cancer patients.

The public's interest in using herbal medicine to seek a cure for the disease is getting higher. People think herbal medicine is safer than medicine made by doctors and does not use synthetic chemicals that can harm health. The Bangka Belitung Islands are one of the areas that have a wealth of herbal plants whose benefits are strongly believed by the local community.

Mentangor plant (*Calophyllum rufigemmatum*) is a plant that grows a lot in Bangka Belitung and has been used by the people of Bangka Belitung as a traditional medicine to treat skin diseases. Based on research conducted by Tanjung et al. (2019) on the bark of Bintangor (Calophyllum tentrapterum Miq.) from East Kalimantan, the bark of Bintangor contains benzofuran, senton, phenicumarin, and active compounds. Another discovery of the star plant found a new active compound, calotetrapterin A-C, and the new compound was tested on leukemia cancer cells (murine leukemia P 388) cells and showed a very active strength [4]. The antioxidant activity of the ethanol extract of the bark of Bintangur (Calophyllum soulattri Burm. F) has a high antioxidant content of 4.29 mmol Fe2+/100 gm [5]. However, the mentangor stem from Bangka Belitung has never been carried out regarding its phytochemical content and potential as an anticancer of the breast. For this reason, the pharmacological effects of breast cancer and phytochemicals on Mentangor Bangka Belitung's stem bark are interesting.

RESEARCH METHODS

Tools and materials

The equipment used in this study were: sample bottles, beakers, measuring cups, test tubes, volumetric flasks, dropper pipettes, volume pipettes, blenders, Petri dishes, funnels, filter paper, stirring rods, analytical balances, rotary evaporator, ELISA Reader, microplate, micropipette, analytical balance, vortex machine, microscope, aluminum foil, and spatula. In comparison, the materials used were mentangor bark, 96% ethanol, methanol,

Mayer reagent, Wagner reagent, FeCl3, Mg solids, Chloroform, H2SO4 2N, glacial acetic acid, cancer cells MCF-7, cisplatin, distilled water.

Sample Preparation

The bark of mentangor (*Calophyllum rufigemmatum*) was obtained from Dendang Village, Kelapa District, West Bangka Regency, Bangka Belitung Islands. In this study, three types of stem bark diameter were used, namely 1 cm, 7 cm, and 17 cm. Furthermore, the sample will be dried in the open air, blended into dry powder, and sieved [6].

Extraction of Active Compounds

In this study, the extraction of the bark of Mentangor stems using the maceration method. The stem bark of each diameter of 100 g was dissolved in ethanol solvent at 1: 10 (g: v). The samples were then left in the dark for three days. Every 1 x 24 hours, vacuum and solvent replacement filtering is carried out. Then the extract solution was evaporated using a rotary evaporator to obtain a thick extract [7].

Phytochemical Test

Phytochemical tests were carried out at the MIPA Laboratory of FPPB UBB to know the secondary metabolites contained in the stem bark of Mentangor at different diameters. Several methods carried out this identification, including tests for tannins, flavonoids, phenolics, alkaloids, steroids, and terpenoids. The tannin test used the iron (III) chloride (FeCl3) method, the flavonoid test used the Wilstater Cyanidin method, and the alkaloid test used the Mayer and Wegner method. In contrast, the steroid test used the Liebermann-Buchnard method [8-9].

Cytotoxic Activity Test

The method used in testing the cytotoxic activity was the microtetrazolium (MTT) test (3-[4,5dimethylthiazol-2yl]2, 5-diphenyltetrazolium bromide). 100 L of the test solution was suspended with 100 L of cells in RPMI 1640 medium plus 10% FBS and 1% penicillin-streptomycin (density 2x104 cells/well) and then put into microplate 96. MCF-7 cells were incubated for 24 hours in an incubator. CO2 5% with suitable humidity. Samples were put in plates with various levels of 1000 g/mL; 500 g/mL; 250 g/mL; 125 g/mL; 62.5 g/mL; and 31.25 g/mL. The samples were then incubated in a 5% CO2 incubator for 24 hours. Live cells will react with the MTT reagent to form a purple color (formazan). The cell medium was then discarded, and 110 L of MTT reagent was added to each well, including the control medium (without cells). After incubation for 4 hours, 100 L SDS 10% was added to stop the reaction between live cells and incubated overnight (24 hours) at room temperature in the dark. At the end of incubation, the absorbance was read with an ELISA reader at a wavelength of 570 nm 12.

Absorbance data for each well, then converted into percent cell viability using the equation:

% viabilitas = (a-b)/(c-b) x 100%

Information:

a = treatment cell absorbance;

b = absorbance control medium;

c = control cell absorbance.

The IC50 value was calculated using a linear regression equation that describes the relationship between the percentage (%) of MCF-7 cell viability with the log concentration of the test sample:

Y=ax+b

Information:

x = compound concentration log;

y = percent cell viability;

IC50 = anti log x [10]

RESULTS AND DISCUSSION

Mentangor Bark Extract

Mentangor bark that has been dried and then mashed into powder. This refinement aims to increase the surface area of the particles. So, the greater contact of the particles with the solvent facilitates the extraction process. Mentangor bark extraction using the maceration method. Maceration of the stem bark samples using ethanol as solvent. Maceration was done by immersing the simplisia of each mentangor bark with a diameter of 1 cm, 7 cm, and 17 cm in ethanol solvent for 3 x 24 hours and stirring every 1 x 24 hours. Each extract was then concentrated with a rotary evaporator to evaporate the solvent so that a thick extract of 20,11 g, 25,43 g; 22,89 was obtained.

Phytochemical Screening Test

Phytochemical identification was carried out qualitatively by observing changes in the test sample. Phytochemical identification aims to determine the content of secondary metabolites in a test sample. The secondary metabolites identified in the qualitative test of the metangor bark extract include alkaloids, flavonoids, steroids, phenolics, and tannins.

A class of phenol hydroquinone compounds (tannins) is known from the results of the FeCl3 test, which shows a green or bluish-green color is formed. It indicates a phenol hydroquinone compound from the ethanol extract of Calophyllum rufigematum. The results of the flavonoid test using the Wilstater Sianidin test method showed flavonoid compounds in the ethanol extract of Calophyllum rufigematum, which was shown in orange color.

The class of steroid compounds was identified by the Liebermannn-Burchard test

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method. The results of this test showed a positive test with the formation of a dark green color. Meanwhile, testing of alkaloids and saponins showed negative test results [11]. The phytochemical test results of ethanol extract calophyllum rufigematum are presented in table 1.

Table 1. Phytochemical Test Results of Ethanol Extract of Mentangor Bark

Test Diameter	Tannins	Flavonoids	Alkaloids	Steroids	Phenolics
1 cm	+	+	+	-	+
7 cm	+	+	+	-	+
17 cm	+	+	+	-	+

Description: (+) = contains active compounds, (-) = does not contain active compounds

Based on the results of phytochemical screening, the results showed positive (+) reactions to several test reagents. The ethanolic extract of the stem bark of Mentangor contains tannins, phenolic compounds, flavonoids, and alkaloids. In comparison, steroid compounds were not found in each extract of the stem bark of Mentangor.

Cytotoxic Activity Test

Cytotoxic activity was tested using the microtetrazolium (MTT) test method. This test aims to determine the magnitude of the cytotoxic potential of the sample against MCF-7 cancer cells in vitro, which is expressed by the IC50 parameter. Test the cytotoxic activity of the stem bark extract at 1 cm, 7 cm, and 17 cm using the MTT method with variations in levels of 1000 g/mL; 500 g/mL; 250 g/mL; 125 g/mL; 62.5 g/mL; and 31.25 g/mL with 3 repetitions for each sample. Extracts were measured with an ELISA reader at a wavelength of 570 nm12. Analysis of the cytotoxic activity of the stem bark of mentangor is presented in Table 2.

Table 2. Cytotoxic Activity Mentangor 1 cm against MCF-7 cells

Concentration (µg/mL)	Concentration Log	Viability (%)	IC_{50} (µg/mL)	Description
31.25	1.495	-3.702	326.195	Moderate
62.5	1.796	21.739		
500	2.699	62.534		
1000	3.000	73.093		

Table 3. Cytotoxic Activity Mentangor 7 cm against MCF-7 cells

Concentration (µg/mL)	Concentration Log	Viability (%)	IC_{50} (µg/mL)	Description
31.25	1.495	4.870	186.178	Moderate
62.5	1.796	14.534		
500	2.699	76.323		
1000	3.000	80.273		

Table 4. Cytotoxic Activity Mentangor 17 cm against MCF-7 cells

Concentration (µg/mL)	Concentration Log	Viability (%)	IC_{50} (µg/mL)	Description
31.25	1.495	-7.304	178,090	Moderate
62.5	1.796	6.833		
250	2.699	58.087		
500	3.000	85.863		

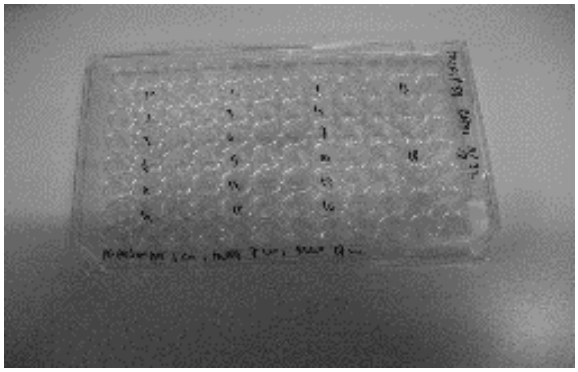

Figure 1. Anticancer Test Template

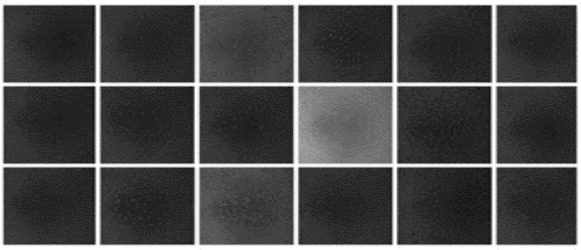

Figure 2. Proliferation Inhibition Development

The results of the measurement of the IC50 value of inhibition of the proliferation of the ethanol extract of the stem bark of Mentangor with a diameter of 1 cm. 7 cm. and 17 cm against MCF-7 breast cancer cells were 326,195 g/mL, respectively; 186.178 g/mL; and 178.090 g/mL. The positive control used was cisplatin with an IC50 value of 8.61 g/mL. The IC50 values of the ethanol extract of the stem bark of mentangor show that the level of cytotoxicity of the stem bark of mentangor is moderate cytotoxicity which can function as a chemopreventive agent which can only inhibit and prevent breast cancer cells. Based on the results of the analysis of the 17 cm diameter mentangor bark extract, it has the potential to be developed as a chemopreventive agent in the treatment of breast cancer, where at a concentration of 500 g/mL, the extract can inhibit cell proliferation by 85%. The cytotoxic ethanol extract of the stem bark of mentangor (*Calophyllum rufigemmatum*) has the potential to be developed as a chemopreventive agent compared to the stem extract of Calophyllum rigidum Miq. which has an IC50 value of 1063.17 g/mL which is not toxic to breast cancer cells [12-16].

Phytochemicals of the stem bark of Mentangor contain very strong antioxidants that function as anticancer chemopreventive and curative. It can assist chemotherapy agents in forming a pro-oxidant effect so that cancer cells increase and cell proliferation can be inhibited [3]. Flavonoids are one of the antioxidants that play a role in inhibiting cancer cells by binding to the death receptor (TNF-R) and Fas-associated Death Domain (FADD), which form the Death Inducing Signaling Complex (DISC) [17-21].

CONCLUSION

The ethanolic extract of the stem bark of Mentangor contains tannins, phenolic compounds, flavonoids, and alkaloids. In comparison, steroid compounds were not found in each extract of the stem bark of Mentangor. The results of the measurement of the IC50 value of inhibition of the proliferation of the ethanol extract of the stem bark of Mentangor with a diameter of 1 cm. 7 cm. and 17 cm against MCF-7 breast cancer cells were 326,195 g/mL, respectively; 186.178 g/mL; and 178.090 g/mL. The IC50 value of the ethanol extract of the stem bark of mentangor with diameters of 1 cm, 7 cm, and 17 cm can be seen that the cytotoxicity of the stem bark of mentangor is moderate cytotoxicity. Mentangor bark extract with a diameter of 17 cm has more potential to be developed as a chemopreventive agent in the treatment of breast cancer, where at a concentration of 500 g/mL, the extract can inhibit cell proliferation by 85%.

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