OPEN ACCESS International Journal of Applied Biology

ISSN : 2580-2410
eISSN : 2580-2119

Bioactivity of Nypa fruticans Leaves as A Candidate for Anticancer Compounds Against MCF-7 Breast Cancer Cells

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Abstract

Breast cancer is the highest incidence of cancer for among women worldwide. The use of anticancer compounds from natural ingredients as cancer therapeutics is to reduce chemotherapy side effects. Indonesia has high biodiversity especially its pharmacologically beneficial such as Nipah. Nipah (Nypa fruticans W.) is a type of palm-shaped mangrove plant which widely found in watershed areas . This study aims to explores the bioactivity of ethanol extract from N. fruticans leaves against MCF-7 breast cancer cells. Screening for bioactivecompounds was performed by Gas chromatography-mass spectrometry (GC-MS). Anticancer pre-screening was assessed by brine shrimp lethality (BSL) test and anticancer activity was assessed by 3-(4,5-Dimethylthiazol-2-cyl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. According of GC-MS analysis showed the presence of 54 phytochemical compounds. Some of them have bioactivity as anticancer including Sitosterol, Tocopherol, and Phytol. The BSLT method showed potent activity with LC50 was 84.25 µg/mL. Anticancer activity using MTT assays results the low IC50 value was 88.77 µg/mL that showed high anticancer activity by growth inhibition in MCF-7 breast cancer cells. This study delivers information that ethanol extract from N. fruticans leaves possesses bioactive compounds could be use as a candidate for anticancer compounds for cancer treatment.

Article History
Received December 8, 2021
Accepted June 14, 2022

Keyword
bioactive compounds;
Nypa fruticans;
breast cancer;
MCF-7 cells;
gas chromatography-mass spectrometry

Introduction

Cancer is an abnormal cell growth and uncontrolled proliferation that will form a tumor mass (Greaves M and Maley CC, 2012). Nearly 90% of cancer-related deaths are caused by metastatic processes that can invade other tissues and cause damage to vital functions (Suhail et al., 2019). Breast cancer is one of the most common cancers in women worldwide, accounting for approximately 627,000 atau sekitar 11,6 % deaths in 2018 (WHO, 2018). In Indonesia, the highest incidence of cancer for women is breast cancer, which is 42.1 per 100,000 population with an average death rate of 17 per 100,000 population, followed by cervical cancer at 23.4 per 100,000 population (Kemenkes RI, 2019). Breast tumors usually start from the ductal hyperproliferation, and then develop into benign tumors or even metastatic carcinomas after constantly stimulation by various carcinogenic factors (Sun, Y. S., et al., 2017).

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Cancer treatment have been carried out, namely through surgery, radiation, chemotherapy, and biological therapy. Cancer treatment using chemotherapy can cause side effects in the form of damage to healthy cells. Some side effects of chemotherapy drugs can induce cardiotoxicity (Akazawa, 2017) and heart failure has been documented in women receiving doxorubicin (Watkins, 2019). The risk of endometrial cancer, stroke, pulmonary embolism, and deep-vein thrombosis is increased in Tamoxifen-treated patients (Yang Y, 2017). In addition, the use of chemotherapy can also cause problems with gene mutations and drug resistance (Fisusi and Akala, 2019). Better medicines with less adverse effects and afavorable risk-benefit ratio need to be developed in the future. Reducing risk factors and taking chemoprevention are two main measures to prevent breast cancer.

One of the plants that can be used is Nipah (Nypa fruticans). Research conducted by (Edu et al., 2015; Ebana et al., 2015; Lestari et al., 2016; Habibi, 2017; Lovly and Merlee, 2018; Ubulom et al., 2019; Gazali et al., 2019) found that Nipah leaves extract contains polyphenolic compounds, flavonoids, triterpenoids/ steroids, saponins, alkaloids, tannins, and cardiac glycosides, with various bioactivities such as antioxidant, anticancer, antimicrobial, antifungal, antiviral, and others.

In addition to anticancer potential, Nipah leaves also have potential as antioxidants.

Nipah leaves contain high concentrations of phenolic compounds and an antioxidant value with an IC50 value of 0.32 mg/ml (Aziz and Jack, 2015). The results of Putri et al. (2012) showed that the antioxidant activity of nipah leaf extract had an IC50 value of 17.72 ppm where the value was close to the standard value of antioxidant vitamin C. Not much different, Lovly and Merlee (2017) reported that the methanol extract of Nipah leaves showed high antioxidant activity with an IC50 value of 6.11 g/ml. Supporting the results of previous studies, the results of the antioxidant activity test of the methanol extract of Nipah leaves showed an IC50 value of 9.31 g/mL (Gazali et al., 2019). Anticancer activity can be related to its antioxidant activity.

One of the causes of cancer is free radicals that attack the cells of the human body. Antioxidants can prevent cells from oxidative damage, which causes cancer and cardiovascular disorders (Muniyandi et al., 2019).

Materials and Methods

Equipment and Materials

The tools used are Ultrasonic Homogenizer Merek Hielscher UP200St, filter paper, Rotary Evaporatory, Vacuum Pump, Chiller, analytical balance, funnel, erlenmeyer, beaker glass, micropipette, dropper pipette, vial bottle, 96-well plate, Biosafety Cabinet (BSC), Centrifuge, CO2 Incubator, Misroscope, Multimode Reader, UV-Vis spectrophotometer, tissue cultureflask, microtube, tube, GC-MS (Gas Chromatography Spectrofotometry Massa) QP2010 Simadzu. The ingredients used are Nypa fruticans leaves powder, ethanol 96 %, Artemia salina larvae, Artificial sea water, Cisplatin, Ceftriaxon, Dimethil sulfoxide (DMSO), Phosphate Buffered Saline (PBS), Prestoblue, Roswell Park memorial Institute Medium (RPMI), Fetal Bovine Serum (FBS), Trypsin EDTA, Tripsin Blue.

Research Prosedures

Nipah Leaf Extraction

Nipah leaf powder samples as much as 300 grams were extracted using 3 liters of 96% ethanol solvent by ultrasonic method at 100% amplitude and 26 Khz frequency for 30 minutes. Extraction was carried out for 30 minutes. Then the filtrate was filtered and obtained about 800 ml. The filtrate was then concentrated with a rotary evaporator (RE) and 70 ml of dark green thick extract was obtained.

Brine Shrimp Lethality Test (BSLT) Method

Preparation of Artemia salina larvae was carried out by incubating eggs placed in bottles filled with artificial seawater prepared by dissolving 35 g of sodium chloride in 1 L of distilled water, for 48 hours until hatching was incubated at room temperature (28-30 °C) under aeration conditions. strong and continuous light, larvae (nauplii) hatch within 48 hours. Adding 10 Artemia salina shrimp larvae to each extract that had been dissolved in dimethyl sulfoxide (DMSO) and prepared at concentrations of 0.01, 0.1, 1, 10, 100, 1000 ppm in 3 replicates for control and each extract solution. After incubation for 24 hours, observations were made under light and counted the surviving larvae.

MTT Method

Prepared 8 pieces of 1.5 mL microtubes, then each microtube was labeled with the appropriate dilution concentration, then the stock sample was diluted into 8 concentration variants using a media solvent. Take out 96 wellplates containing cells from the incubator.

Labeled on the plate along the left margin for which rows will be standardized and which rows will be sampled. Then removed the media from each well. Using a micropipette, transfer 100 L of each sample and positive control of cisplatin from the microtube into each of the corresponding wells, 96 well plates containing cells. Incubate for 48 hours. Discarded media on each well. Prepare 9 ml of media in a tube, add 1 ml of PrestoBlue Cell Viability Reagent then add 100 l of the solution mixture into each well of the microplate and then incubate for 1- 2 hours until changes are seen color.

GC-MS Analysis

The ethanol extract of Nipah leaves was analyzed using GCMS QP2010 Simadzu. Identification of phytochemical compounds using the Willey database version 7.0 by comparing the mass spectrum pattern and the fragmentation pattern of reference compounds stored in the Willey library.

Results and Discussion

The results of toxicity test using the Brine Shrimp Lethality Test (BSLT) method was shown in Table I. Table I shows that the higher the sample concentration, the higher the mortality percentage of Artemia salina larvae. The lowest concentration of Nipah leaf extract, 1 g/mL, gave a mortality effect on Artemia salina larvae of 13%, while the highest concentration of Nipah leaf extract of 100 g/mL gave a mortality effect on Artemia salina larvae of 50%. The results showed potent activity with LC50 was 84.25 µg/mL, included in the toxic category and has potential as an anticancer compound.

Table 1. The results of the toxicity test using the Brine Shrimp Lethality Test method.

Sample Code	[Sample Concentration] (µg/mL)	Axis x (log [Sample])	% Kematian larvae - control	Axis y (Probit Value)
Nipah	1	0,00	13	3,87
Leaves	10	1,00	33	4,56
Extract	100	2,00	50	5,00

The Brine Shrimp Lethality Test (BSLT) is a preliminary test to determine the toxicity of a compound or extract acutely using shrimp larvae experimental animals (Artemia salina nauplii). Parameter shown to indicate the presence of biological activity in a compound in Artemia salina Leach is the number of shrimp larvae mortality due to the influence of administration of the compound at a predetermined dose (Kurniawan and Ropiqa, 2021). The low value of LC50 of BSLT method has been evidenced of the presence of anticancer compounds in plant extracts (Geethaa S., et al., 2013).

The test results of MCF-7 breast cancer cells using the MTT method was shown in Figure I. Ethanol extract from Nipah leaves has the low value of IC50 about 88.77 µg/mL. It states that viability of MCF-7 cells will decrease in line with increase or decrease in extract concentration from a concentration of 88.77 µg/mL.

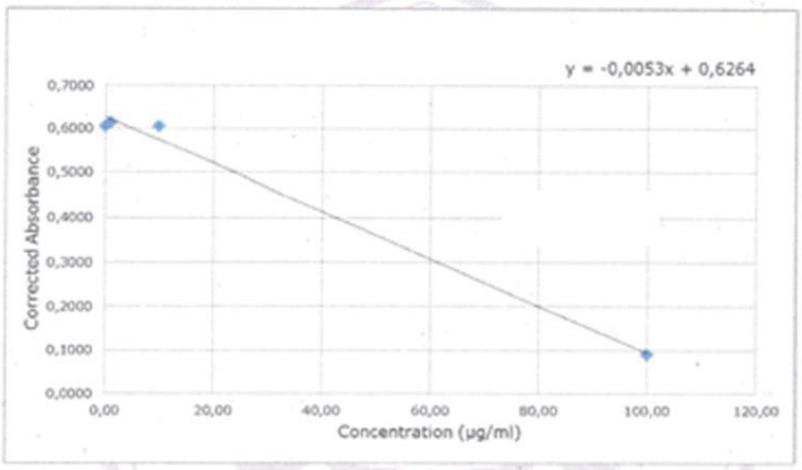

Figure 1. Cytotoxicity graph of Nipah leaves ethanol extract against MCF-7 cancer cells

These results indicate that the higher the concentration of Nipah leaves extract, the smaller the percentage of live cells. Based on Figure 1, showed that as the concentration of the sample increased, the number of living cells decreased. The IC50 value of 88.77 µg/mL was obtained and was included in the very toxic category, which means that it has the potential to inhibit the growth of MCF-7 breast cancer cells and also potential as candidate for anticancer compounds. This is supported by the presence of bioactive compounds which, based on previous research, can act as anticancer compounds.

Ethanol extract of Nipah leaves possessed many bioactive compounds. GC-MS analysis

revealed the presence of more than fifty four compounds, and some of them found in larger quantity than other compounds which showed in Table 2.

Tabel 2. The majority of phytochemical compounds contained in the ethanol extract of Nypa fruticans using the GC-MS method

Retention Time	Compound Name	% Peak Area
11,554	Phytol	8.54
18,699	Tocopherol	7.01
20,604	Sitosterol	12.11

In Table 2, it can be seen that the phytochemical compounds that have been identified in the ethanol extract of Nypa fruticans leaves by GC-MS method include sitosterol, tocopherol, and phytol. From the results of the chromatogram (Figure 2), there is one compound whose composition is larger (indicated by % total the largest, 12.11%) was sitosterol.

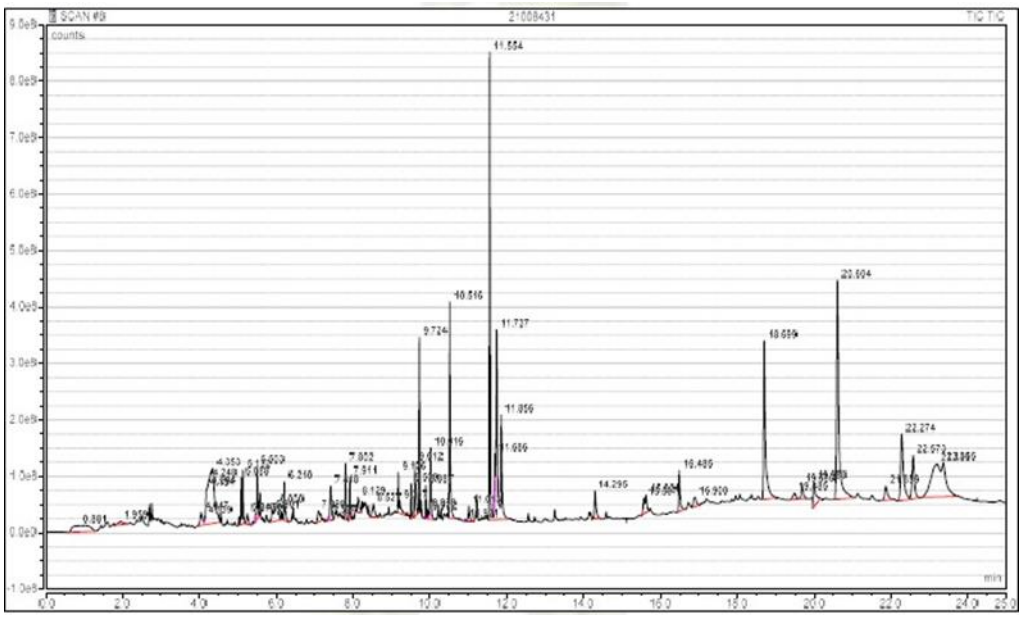

Figure 2. GC-MS chromatogram of Nypa fruticans ethanol extract

Sitosterol is a phytosterol widely distributed throughout the plant and its function to be involved in the stabilization of cell membranes. Sitosterol can treating different types of cancer via different pathways (Bin Sayeed, M.S. and Ameen, S.S., 2015). Sitosterol function was could inhibits the growth of cultured cancer cell lines that are associated with stimulation of apoptotic cell death (Awad, A., et al., 2007), inhibition of cancer cell proliferation at low concentrations with no cytotoxic effect on noncancerous cells (Jayaprakasha, G., 2007), arresting of cells at G2/M phase in cancer cells and decreasing free radical generation in vitro (Shahdaat M., et al., 2016). Dietary sitosterol supplementation could decreased mammary hyperplastic lesions and total tumor burden in female mice fed with a high-fat diet but not in those with a low-fat diet (Salehi B., et al., 2020).

α-tocopherols (Vitamin E) have the role, as a potent antioxidant is to uphold the legitimacy of long-chain fatty acids (unsaturated) in superficial surface (cell membrane) and thus sustaining a normal genetic commotion (Batool1 M., et al., 2020). They scavenge lipid peroxyl radicals by donating hydrogen from the phenolic group on the chromanol ring.

Alpha- tocopherol mainly inhibits the production of new free radicals. Oxidation has been linked to numerous possible conditions/diseases including: cancer, ageing, arthritis and cataracts. Thus, tocopherols (Vitamin E) might help prevent or delay the chronic diseases associated with reactive oxygen species molecules (Rizvi S., et al., 2013; Jiang, 2014).

Phytol is terpenoid compounds found in leaf extract. Phytol produced the removal of OH·, exhibiting antioxidant activity which may be capable of inhibiting cell damage caused by this radical (Carolina, et al. 2013). Many papers indicate also that phytol (1) exhibits cytotoxic potential against certain cancer cell lines: leukemia (MV4-11 and HeLa), breast (MCF-7), prostate (PC-3) and lungs (A-549)37–39 and is the substance promising for the treatment of cancer (Gliszczyńska, et al., 2021). Phytol induces apoptosis and reactive oxygen species- mediated protective autophagy in human gastric adenocarcinoma cells (Song and Cho, 2015).

Conclusions

Based on the results of the study, it can be concluded that Nipah leaves extract has potential as an anticancer compounds which is supported by the results of the BSLT method and the MTT method with an IC50 value which is categorized as toxic against MCF-7 breast cancer cells. The toxic nature of nipah leaf extract is due to the presence of bioactive compounds that have antioxidant and anticancer activity such as sitosterol, tocopherol, and phytol.

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