Short communication

Anticancer activities of *Sterculia quadrifida* R.Br bark extract from Semau island, Indonesia on breast cancer cells line T47D

Rr. Listyawati Nurina, Ika Febianti Buntoro, SMJ Koamesah, Muhajirin Dean, Conrad Folamauk

Faculty of Medicine and Veterinary Science, Universitas Nusa Cendana, Indonesia

(Received: June 2023 Revised: October 2023 Accepted: October 2023)

Corresponding author: **Rr. Listyawati Nurina.** Email: listyawati_nurina@staf.undana.ac.id

ABSTRACT

Introduction and Aim: Breast cancer is characterized by the abnormal growth of malignant cells within breast tissue, which can originate from either the ductal epithelium or its lobules. The *Sterculia quadrifida* R.Br plant has been widely used by the urban community as a medicine for jaundice, ulcers, stamina enhancer, postpartum antiseptic and several other indications of disease. The plant contains a class of flavonoids, alkaloids, terpenes and saponins. The objective of this study is to assess the anti-cancer activity of the ethanolic extract of *Sterculia quadrifida* R.Br from Semau Island, Kupang Regency, Indonesia, specifically on T47D cells, and to determine the IC_{50} value.

Materials and Methods: The design of this study is experimental which employed the extraction process, maceration using 96% ethanol. After 24 hours, it was filtered to obtain 96% ethanol filtrate. Additionally, the compound groups present in the extract were characterized through visual observations under visible light, UV light at 254 nm, UV light at 365 nm, as well as visualization using various spray reagents including Dragendorff, $FeCl_3$, 2,4-DNPH, vanillin-sulfuric acid, anisaldehyde-sulfuric acid, and cerium sulfate.

Results: Notably, changes in the morphology and color of T47D cells were observed following their exposure to the 96% ethanol extract *of Sterculia quadrifida* stem bark. The IC50 value analysis in the cytotoxic test involved converting the absorbance data obtained from the test into percentages representing cell viability. Furthermore, the IC50 value, which quantifies the concentration of the extract required to inhibit the growth of T47D cells by 50%, was determined to be 211.4 µg/ml.

Conclusion: In conclusion, it can be inferred that the 96% ethanol extract of *Sterculia quadrifida* stem bark exhibits anti-cancer properties against T47D cells.

Keywords: *Sterculia quadrifida;* breast; cancer; T47D cells.

INTRODUCTION

Breast cancer is a malignancy that originates from either the ductal epithelium or its lobules within breast tissue (1). According to data from the World Health Organization (WHO) in 2018, breast cancer was the most commonly diagnosed cancer in Indonesia, accounting for almost 17% of the 348,809 total cancer cases. In 2020, according to Global Burden of Cancer data, there were 68,858 new cases of breast cancer in Indonesia with more than 22,000 deaths reported (2).

According to information provided by the Indonesian Health Ministry, the incidence of tumours and cancer in Indonesia has seen a rise, increasing from about 1 case per 1000 population in 2013 to almost 2 cases per 1000 population after 5 years. One of the problems that often arise in the treatment of cancer is chemotherapy drug resistance (drug resistance) (3). The chemotherapeutic agent that is often used in breast cancer therapy is cisplatin (4). Cisplatin causes side effects including: neurotoxicity, nephrotoxicity (5) and bone marrow suppression. Furthermore, there have been reports indicating that the administration of cisplatin has led to the development of resistance. This resistance to cisplatin primarily arises due to alterations in cellular uptake, enhanced drug expulsion, suppression of apoptosis, and heightened DNA repair mechanisms. The resistance observed in cancer cells, as well as the adverse effects associated with cisplatin, are often linked to its utilization at elevated doses aimed at achieving more potent treatment outcomes (6). Therefore, it is necessary to do research to find drugs against breast cancer that are effective and selective.

One of the plants that can be used as an anticancer candidate is *Sterculia quadrifida* R.Br from Semau Island, Kupang Regency, Indonesia. The *S. quadrifida* R.Br plant has been widely used by the urban community as a medicine for jaundice, ulcers, stamina enhancer, postpartum antiseptic and several other indications of disease. The plant contains a class of flavonoids, alkaloids, terpenes and saponins. *S. quadrifida* R.Br originating from Semau Island, Kupang Regency, Indonesia has more organoleptic with a more striking red color and more astringent taste compared to *Sterculia* from other areas, thus it is possible that *S. quadrifida* R. Br comes from Semau Island, Kupang Regency, has a strong antioxidant content.

Previous research has established that the ethyl acetate fraction is the most potent, with an EC50 value of 24.88 µg/ml, and it exhibits a high level of selectivity for cancer cells over normal cells, as evidenced by a Selectivity Index value of 15.15. This fraction, derived from the ethanolic extract of *Sterculia quadrifida* stem bark, has demonstrated the ability to arrest the cell cycle in T47D breast cancer cells and enhance apoptosis induction in these cells. Additionally, a fraction obtained through preparative thin-layer chromatography has shown significant antioxidant properties, with an EC50 value comparable to that of vitamin C. It is important to note that the scientific literature regarding the efficacy of *S. quadrifida* bark remains limited, and the potential of *S. quadrifida* stem bark has not been extensively explored. Therefore, this study aims to assess the anticancer activity of the ethanol extract of *S. quadrifida* bark stem from Semau Island, Kupang Regency, Indonesia, particularly in the context of T47D breast cancer cells. The aim is to identify the active fraction responsible for these effects and contribute to the scientific understanding of *S. quadrifida*'s potential in cancer treatment.

MATERIALS AND METHODS

The chemicals and materials used in this study for extraction, phytochemistry, and thin-layer chromatography include acetone, n-hexane, diethyl ether, ethyl acetate, methanol, ethanol, distilled water, Dragendorff reagent, Mayer reagent, Liebermann-Burchard reagent, FeCl3, and sodium hydroxide, as well as silica plate GF254 gel, 60 F silica gel, and glass wool. The cancer cells employed in this research have p53 gene defects (T47D cells). These cells were cultured in complete media, specifically DMEM culture medium for T47D cells, containing 10% FBS (Gibco), 1% penicillin-streptomycin (Gibco), and 0.5% fungizone (Gibco). Cell cultures were maintained in a CO2 incubator, and harvesting involved the use of PBS (Phosphate Buffer Saline) and trypsin. DMSO was used as the solvent for the samples.

For cytotoxicity testing, the materials used included [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) at a concentration of 5 mg/mL and a stopper reagent (sodium dodecyl sulphate, Merck) at 10%.

Various equipment was utilized in this study, including glassware, UV lamps with wavelengths of 254 nm and 366 nm, cups, vials, microtubes, autoclaves, conical tubes, 96-well plates, 6-well plates, tissue culture flasks, 6 cm dishes, an Elisa reader (SLT 340 ATC), falcon flask, analytical balance, centrifuge, incubator set at 37ºC with 5% CO_2, refrigerator, laminar airflow class II cabinet, liquid nitrogen tank, hemocytometer, object glass, cover glass, contrast microscope (Olympus), micropipette, digital cameras, ovens, hammer mills, 40 and 60 mesh screens, desiccators, and porcelain dishes.

The extraction method used was maceration using 96% ethanol. ± 2000 grams of skin powder each soaked in 96% ethanol for 48 hours with frequent stirring. Furthermore, the 96% ethanol filtrate was separated from the residue by filtering. The residue obtained was then soaked again with 96% ethanol for 24 hours while stirring frequently. After 24 hours, it was filtered to obtain 96% ethanol filtrate. This was done repeatedly until a clear filtrate was obtained. The filtrate was evaporated using a rotary evaporator at ±40°C so that a concentrated extract was obtained and then the weight of the 96% ethanol viscous extract was weighed.

Characterization of the compound groups in the extract was carried out by observation under visible light, UV light 254 nm, UV 365 nm and visualization with several spray reagents. The spray reagents used included: Dragendorff, FeCl3, 2,4-DNPH, vanillin-sulfuric acid, anisaldehyde-sulfuric acid, and cerium sulfate. After treatment with spray reagent, the following observations were made: 1) Cerium sulfate reagent: after being sprayed, the plate was heated and observed under visible light; 2) Anisaldehyde-sulfuric acid, vanillin-sulfuric acid and 2,4-DNPH reagent: after spraying, the plate is heated at 105 ºC for 5 minutes, observed under visible light; 3) Dragendorff reagent and FeCl3: after being sprayed the plates were observed under visible light without heating (8).

Data analysis

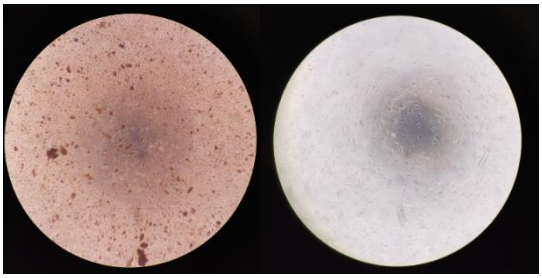
The IC50 value analysis in the cytotoxic test involved converting the absorbance data obtained from the test into percentages representing cell viability. These percentages were calculated. Subsequently, the data, now in the form of cell viability percentages, were analyzed using the Microsoft Excel 2016 program. This analysis aimed to establish the linearity (R) between the logarithm of the concentration and the percentage of viable cells and to calculate the IC_{50} value.

RESULTS

Extraction of plant samples

From the extraction which was carried out using dry powder of *S. quadrifida* stem bark from Semau Island as much as 30 g with 96% ethanol solvent, 2.1 g of dry extract was obtained.

Cytotoxicity test: The cytotoxicity test used the ethanol extract of *S. quadrifida* stem bark from Semau Island with the target cells being T47D breast cancer



(a) (b)

Fig. 1: Photo of test results of 96% ethanol extract of *S. quadrifida* stem bark against T47D breast cancer cells. (a) T47D cells before treatment, (b) T47D cells after treatment

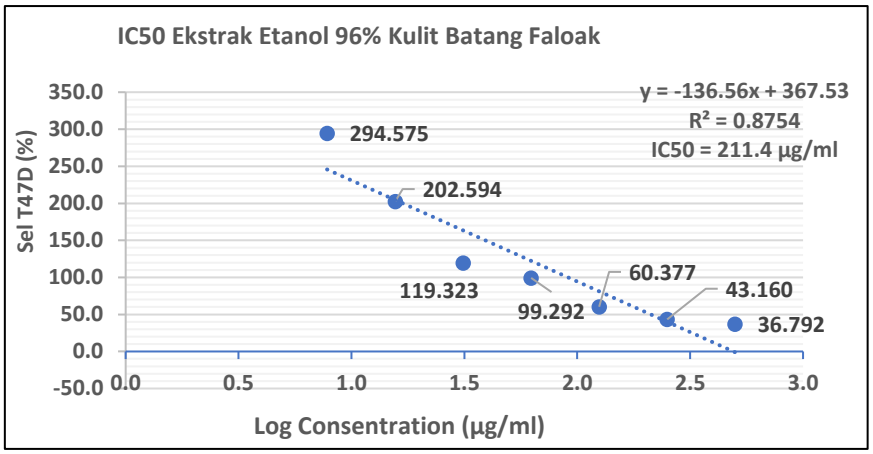


Fig. 2: IC_{50} Ethanol extract 96% of *Sterculia quadrifida* stem bark against T47D breast cancer cells

cells with the following results: The results obtained from the two above were changes in the shape and color of the cells after being given 96% ethanol extract of *S. quadrifida* stem bark (concentration of 250µg/ml). The cells in the image change color from white to brick red and T47D breast cancer cells appear (Fig. 1).

From Fig. 2, the regression equation is determined which is obtained from the calculation results from the log concentration (µg/ml) as the x-axis and T47D breast cancer cells as the test target. The straight-line equation obtained is y = -136.56x + 367.53. Then do the calculations for the IC_{50} value, the value then the IC50 value is obtained = 211.4 µg/ml.

DISCUSSION

The *Sterculia quadrifida* R. Br plant is a medicinal plant used by the people of East Nusa Tenggara Province, especially Kupang City (western part of Timor Island) as medicine. For this study, the stem bark used was the *S. quadrifida* plant from Semau Island. The choice of the *S. quadrifida* plant from Semau Island was due to the fact that previous research had not been carried out either from extraction and anticancer activity testing of the breast and the *S. quadrifida* plant has a different habitat than on Timor Island, which is located on the beach.

The *S. quadrifida* stem bark was extracted using 96% ethanol solvent because the active substance contains properties that are easily soluble in polar solvents. This is based on empirical use by people who use water as a solvent in daily medication. 96% ethanol is used because it is polar and prevents the growth of microbes, so the extract obtained is more durable. Extraction was carried out using 30 g of dry powder of *S. quadrifida* stem bark then maceration was carried out using 96% ethanol solvent to obtain a dry extract of 2.1 g (9).

In addition to the *S. quadrifida* plant used, T47D breast cancer cells were also used as a test target because the same test had been carried out before and the extract of the *S. quadrifida* plant was obtained. T47D cells are breast cancer cells that have characteristics of caspase 3 wildtype, caspase-7 wildtype, ER/PR positive and p53 mutant so that they can be used as cell lines for testing cell apoptosis (anti-cancer) (10). The results of the anticancer (apoptosis) test obtained (Fig. 2) showed the death of T47D cells exposed to 96% ethanol extract of *S. quadrifida* stem bark which was characterized by the presence of damaged cells that did not resemble T47D cells prior to exposure. The brown color observed in the observations was caused by 96% ethanol extract which had a brown color and attached to damaged T47D cells.

The concentrations of the 96% ethanol extract of faloak stem bark used were 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml, 15.625µg/ml and 7.8µg/ml. This concentration variation was carried out based on previous research which obtained an EC_{50} value of 182.42µg/ml (11) which was the median value in determining IC_{50} in this study.

From the observation of T47D cells, it was found that 96% ethanol extract of *S. quadrifida* stem bark could inhibit or cause death of T47D breast cancer cells. Subsequently, a calculation (50% inhibitory concentration / IC_{50}) was performed to see at what concentration the 96% ethanol extract of *S. quadrifida* stem bark could cause 50% cell death (apoptosis) T47D. Calculation of IC_{50} can be done using a linear regression equation. The linear regression equation obtained is $y = -136.56x + 367.53$ so that an IC_{50} value of = 211.4 µg/ml is obtained.

CONCLUSION

Based on this study, it can be concluded that the 96% ethanol extract of *Sterculia quadrifida* stem bark has anti-cancer activity on T47D cells. In addition, the IC_{50} value obtained is 211.4 µg/ml.

ACKNOWLEDGEMENT

The Universitas Nusa Cendana, Indonesia, fully funded and supported this research.

CONFLICT OF INTEREST

There is no conflict of interest found during this study.

REFERENCES

1. Harbeck, N., Penault-Llorca, F., Cortes, J., Gnant, M., Houssami, N., Poortmans, *et al.,* Breast cancer. Nature Reviews Disease Primers. 2019;5(1):1-31.
2. Wong, H.L., Bendayan, R., Rauth, A.M., Xue, H.Y., Babakhanian, K., Wu, X.Y. A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer-lipid hybrid nanoparticle system. Journal of Pharmacology and Experimental Therapeutics. 2006;317(3): 1372-1381.
3. Dubey, A., Yadav, P., Verma, P., Kumar, R. Investigation of proapoptotic potential of *Ipomoea carnea* leaf extract on breast cancer cell line. Journal of Drug Delivery and Therapeutics. 2022;12(1): 51-55.
4. Chen, H., Yang, J., Yang, Y., Zhang, J., Xu, Y., Lu, X. The natural products and extracts: anti-triple negative breast cancer *in vitro*. Chemistry and Biodiversity. 2021;18(7): 12-24.
5. Rajendra, C.H., Kedari, G.S.R. Role of antioxidant levels and lipid peroxidation in patients with breast cancer. Biomedicine. 2020;40(2): 170-174.
6. Kour, M., Bhat, K.M.R., Anatharaya, V.N., Chakraborty, S., Chandra, R.K. Efficacy of *Vitex agnus* in lowering prolactin level in mammary tumor induced SD rats. Biomedicine. 2019; 39(2):380-386.
7. Usha, B., Jyothsna, K. Antioxidant, anti-inflammatory, analgesic and anti-proliferating activities of *Grewia heterotricha* Mast. Biomedicine, 2020;40(3):272-277.
8. Gomathi, A.C., Rajarathinam, S.X., Sadiq, A.M., Rajeshkumar, S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line. Journal of Drug Delivery Science and Technology. 2020; 55:101376-101389.
9. Tabrez, S., Khan, A.U., Hoque, M., Suhail, M., Khan, M.I., Zughaibi, T.A. Biosynthesis of ZnO NPs from pumpkin seeds' extract and elucidation of its anticancer potential against breast cancer. Nanotechnology Reviews. 2022;11(1):2714-2725.
10. Waks, A.G., Winer, E.P. Breast cancer treatment: a review. JAMA. 2019;321(3): 288-300.
11. Gharari, Z., Hanachi, P., Walker, T.R. Green synthesized Ag-nanoparticles using *Scutellaria multicaulis* stem extract and their selective cytotoxicity against breast cancer. Analytical Biochemistry. 2022;653: 114786-114796.