

# Cytotoxic Effect of Extract *Aaptos suberitoides* Marine Sponge in MDA-MB 231 Triple Negative Breast Cancer Cell Line

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## Abstract.

**Background :** Triple negative breast cancer (TNBC) is a type of breast carcinoma with the worst clinical manifestations and prognosis and has high potential for distant metastasis. *Aaptos suberitoides* is one of many marine sponges found in Indonesian waters that contain *aaptamine* and possess anticancer effect.

**Objective :** Currently there are no effective therapy for TNBC, hence this study aims at the development of alternative therapy as complementary therapy in triple negative breast carcinoma's standard treatment.

**Material and Method :** Experimental study is performed on MDA-MB 231 cell line that has been given marine sponge *Aaptos suberitoides* extract to see cytotoxic effect through MTT assay and microscopic observations. The IC<sub>50</sub> was analyzed using GraphPad Prism 7.

**Result :** The marine sponges *Aaptos suberitoides* extract demonstrated cytotoxic activities with an IC<sub>50</sub> value of 6 ppm in MDA-MB 231.

**Conclusion :** There is a potential cytotoxic activity of *Aaptos suberitoides* in triple negative breast cancer cell line. These results shows that the administration of *Aaptos suberitoides* extract is feasible to consider as a candidate for TNBC's complementary therapy.

**Keywords :** *Aaptos Suberitoides*, Cytotoxic assay, Marine sponges, TNBC.

## INTRODUCTION

Breast cancer is the most common cancer in women worldwide.[1] Molecular classification of breast cancer based on immunohistochemical assay of *estrogen receptor* (ER), *progesterone receptor* (PR), *human epidermal growth factor receptor* (Her2) and Ki67 were divided into 5 subtypes: Luminal A, Luminal B HER2 negative, Luminal B HER2 positive, HER2 *enriched*, and *Basal Like* or better known as *Triple Negative Breast Cancer* (TNBC).

TNBC is a breast carcinoma with the worst clinical manifestations and prognosis and shows aggressive behavior with high potential for distant metastasis compared to other breast cancer subtypes. 15-20% of breast cancer including TNBC.[2] Often times, TNBCs are resistant to cytotoxic chemotherapy and causes difficulties in achieving personalized medicine due to its molecular heterogeneity.[3] Typically, patients with TNBC tend to have a higher chance of early relapse after diagnosis, short disease-free intervals, and reduced overall survival.<sup>4</sup> Women with TNBC subtypes have a high percentage of recurrence rate in the first three years (34%). [4]

According to Globocan 2018, breast cancer is a malignancy found in women that occupies the second leading causes of death in the world. Data presented by the Ministry of Health as of January 31<sup>st</sup> 2019 shows that there are 42.1 breast cancer cases per 100,000 population with an average death rate of 17 per 100,000 population and its incidence rate as 21.4 % of all malignancies in women in Indonesia.[5] While, triple negative breast carcinoma in Hasan Sadikin Hospital, Bandung, Indonesia in 2015 to 2019 account for 11,6 % of all breast malignancies.

Up to now, TNBC only treated with a combination of surgery, radiation therapy, and chemotherapy and because the genes associated with TNBC are not well understood, for now, there is no target therapy for TNBC yet.[6] TNBC's aggressive behaviour, poor prognosis, and the absence of its effective therapy led to the development of alternative medicine complementary therapy in the standard treatment of triple negative breast carcinoma. It has been suggested that metastatic cancer cells may avoid cell death through the induction of autophagy.[7]

Marine natural products have an important role in the discovery of instructions for the development of medicines for the treatment of human diseases.[8] Indonesia is the largest archipelago country in the world with the second longest coastline in the world and is the richest country in the world in terms of the diversity of marine organisms.[6]

*Aaptos suberitoides* is one of many marine sponges found in Indonesian waters.[9] Previous study found *aaptamine*, bioactive compound in *Aaptos suberitoides*, have proapoptotic and anti-proliferation effects by modulating the transcription factor of cell growth regulator protein such as *Activator protein 1* (AP-1), *Nuclear factor kappa-light-chain-enhancer of activated B cells* (NF-κB), and *Protein 53* (p53) in fibrosarcoma cell.[10] A study revealed that *Aaptos suberitoides* contains *aaptamine* component which possess cytotoxic effect on HeLa cells.[11] Another study also revealed that *Aaptos suberitoides* contains a significant anti-cancer effect on *murine lymphoma* cell line.[12] To date, there is no previous study which evaluates the effects of *Aaptos suberitoides* in triple negative breast cancer cell is published.

Therefore, this study aim to evaluate anti-cancer potential of the *Aptos suberitoides* in triple negative breast cancer by cytotoxic activity.

#### METHOD

The research was conducted at the Histopathology and Immunohistochemistry Laboratory, Anatomy Pathology Department, Faculty of Medicine, Padjadjaran University, Dr. Hospital. Hasan Sadikin Bandung and in the Cytogenetic Cell and Culture Cell Laboratory of RSP Unpad Jalan Prof. Eyckman No. 38 Bandung.

#### Material

The materials and instruments used are 96% ethanol, culture media RPMI1640 (*Gibco, cat.no. 11875085, USA*), *Fetal Bovine serum (FBS) (Gibco, cat.no. 26140079, USA)*, *penicillin/Streptomycin (Sigma, cat.no. P4333, USA)*, *phosphate buffered Saline (PBS) (gibco, cat.No. 10010001, USA)*, *trypsin (gibco, cat.No. 15050057, USA)*, *dimetil sulfoksida (DMSO) (Sigma, cat No. D8418, USA)*, cell culture incubator (*Thermo Scientific model 3429, USA, 2014*), *Inverted Microscope (Olympus CK40, Japan)*, microscope camera (*C-Mount camera, China*), *imagej Software (v 1.51, National Institute of Health, USA)*, *Microsoft Excel software (v. 16.30, 2016)*, *GraphPad Prism Software (v 7.0 a, 2016)*.

#### Cell Culture's Condition

MDA-MB 231 cells were used as model for *triple negative* breast carcinoma. This cell was obtained from Dr. Wiemann (National Center for Tumor Diseases, Heidelberg, Germany). The status of this MDA-MB 231 cell is IHC ER/PR and Her2 negative. This cell was cultured using RPMI 1640 equipped with 10% FBS and 1% penicillin/streptomycin in a standard incubator maintained at the temperature of 37 ° c and 5% CO<sub>2</sub>. All experiments were carried out in the cell culture laboratory and *Cytogenetic*, Padjadjaran University teaching hospital on Prof. Eyckman street No. 38 Bandung.

#### MTT Assay

*Aptos suberitoide's* toxicity effects were evaluated using MTT assay. Cells were planted in 96 well plates, induced for 24 hours, and treated with serial ethanol concentration of *Aptos suberitoides* extract and then followed for 72 hours. After that, cells were treated with MTT reagents for 4 hours and the reaction was stopped using DMSO. Then,

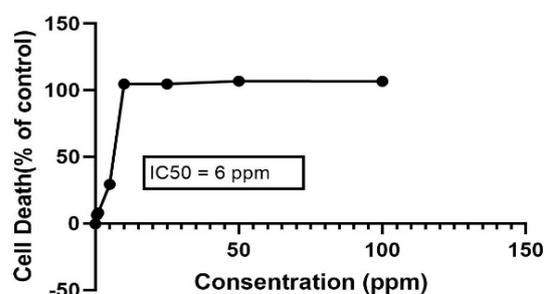
the plate is shaken to dilute the *crystal formazan* before its absorbance is read using a reading plate with a wavelength of 550 Nm. The percentage of cell death was calculated based on the absorbance values of the sample, control, and blank. The experiment was carried out in 3 repetitions with serial concentrations doubled.

#### Morphological Observation

In addition, to evaluate the morphological changes of MDA-MB 231 cells, the cells were seeded on cover slip of 24 well-plate followed treated by concentration 5 ppm of the extract. Cells were captured under a light microscope connected with camera at 200x magnification and observed for the morphological characteristics of cell death.

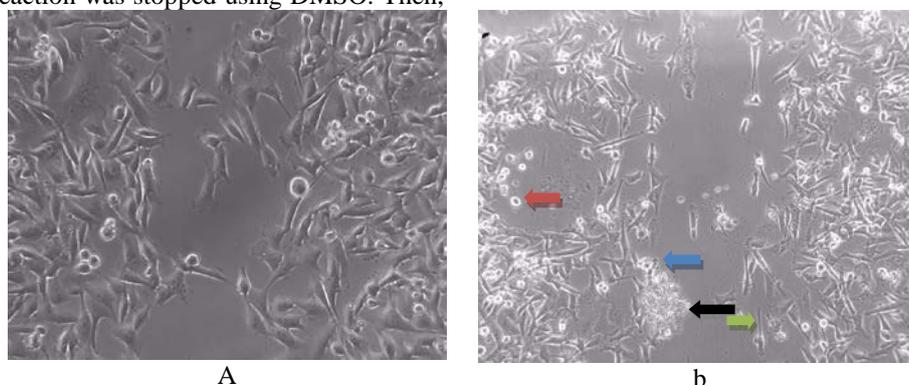
#### RESULT

Data showed that the extract of *Aptos suberitoides* induced cell death in triple negative breast cancer cell line. In addition, the cytotoxic activity extract of *Aptos suberitoides* was shown in dosed-dependent manner. The IC<sub>50</sub> value was 6 ppm. (Fig. 1)



**Figure 1.** Cell death curve of MDA-MB 231 upon treatment of extract of *Aptos suberitoides*

Morphological examination of MDA-MB 231 line cells given *Aptos suberitoides* extract treatment with a concentration of 5 ppm compared to control (not treatment). Data showed that the ethanol extract of *Aptos suberitoides* induced morphological changes of MDA-MB 231 cells including cell shrinking and rounded, apoptotic bodies as well as vacuole formation and pyknosis, indicating that this extract triggered cell death in triple negative breast cancer cell line refer to apoptotic process. (Fig.2)



**Figure 1. Microscopic Appearance.** The picture showing the microscopic appearance of MDA-MB 231 Cells of untreated (negative control ) (a) and treated with extract of *Aptos suberitoides* with the following concentrations: 5 ppm (b), (magnification 200x). Cells shrinking and rounded (red arrows), the formation of apoptotic bodies (black arrows), and pyknosis (green arrows), vacuole formation (blue arrows).

## DISCUSSION

TNBC's metastasis happens through a very complex process and still not entirely understood yet. This includes several steps such as genetic and epigenetic changes, angiogenesis, tumor and stroma interaction, intravasation via basement membrane, cell migration, survival in circulation, and extravasation to distal tissue.[13] Patients with TNBC have a relatively poor therapeutic responses.[14] Cancer cell metastasis is a complex process that involves proteolytic degradation of the extracellular matrix (ECM), cell migration, adhesion, and invasion, leading to high mortality rates.[15]

TNBC's profile includes response on target therapy, anti Her2 and very low endocrine therapy. Thus, the development of potential alternative therapy to become complementary therapy in TNBC's standard therapy is needed. The aim is to increase the survival of TNBC patient.

Based on previous study showed that extract of *Aaptos suberitoides* contain alkaloid chemical compounds identified as aaptamine (benzol-1,6naphthyridin), demethylaaptamine, and isoaptamine.[10]

Research from Nurhayati et al., stated that *Aaptos suberitoides* marine sponge induces apoptosis in luminal subtype B carcinoma.[16] Tsukamoto et al., reported *Aaptos suberitoides* has cytotoxic effect on cervix carcinoma.[17] Abdillah et al., also reported that *Aaptos suberitoides* marine sponge has anti-proliferation effect on H-29, T47D and Casky cell line.[18] This is in accordance with previous studies, MTT test on MDA-MB 231 cell lines given *Aaptos suberitoides* extract obtained IC<sub>50</sub> results of 6 ppm. The American National Cancer Institute (NCI) guidelines set the limit of activity for crude extracts 50% inhibition (IC<sub>50</sub>) of proliferation of less than 30 µg/mL in 72 hours incubation. Moreover, a crude extract with IC<sub>50</sub> less than 20 µg/mL is considered strong cytotoxic.[19] Cytotoxicity test on each cell line is different. In a study conducted by Nurhayati et al, the cytotoxic activity of the *Aaptos suberitoides* ethanol extract on IC47 T47D (breast carcinoma) cell lines was 153.109 µg / mL.[20] Blowing et al. states that aaptamine compounds have powerful cytotoxicity because of their ability to intercalated DNA. DNA.[11] An IC<sub>50</sub> value below this stringent value was noted for MDA-MB 231 which falls within the NCI criteria thus to be considered as a promising anticancer potential.

Tsukamoto et al. stated that *aaptamine* contained in *Aaptos suberitoides* and its derivative functions as proteasome inhibitors.[11] *Ubiquitin proteasome pathway* is the main component from protein degradation machine inside eukaryotic cell. More than 80% of protein from degraded cell will pass through this pathway, including protein used in apoptosis, transcription, DNA repair and antigen presentation.<sup>7</sup>

This is consistent with research conducted by Dyshlovoy SA et al., Which states that sea sponge *Aaptos suberitoides* has aaptamine compounds that have proapoptotic and anti-proliferation effects by modulating the transcription factors AP-1, NF-κB in monocytic

leukeumia, colon cancer, cervical cancer and breast cancer effects.[21]

Cell death across cell types and species both in the nucleus and cytoplasm has very similar morphological changes.[22] Morphological hallmarks of apoptosis in the nucleus are chromatin condensation and nuclear fragmentation, which are accompanied by rounding up of the cell, reduction in cellular volume (pyknosis) and retraction of pseudopods.[23] The beginning of chromatin condensation begins at the periphery of the nuclear membrane, forming a crescent moon or ring-like structure. Furthermore, chromatin condenses until it ruptures inside the cell with the membrane intact, features described as karyorrhexis.[22] Some morphological features such as blebbing membranes, ultrastructural modification of cytoplasmic organel and loss of membrane integrity are the next stages of apoptosis.[23] In this study, morphological changes from cel line MD-MB 231 extracted by *aaptos suberitoides* saw cell shrinking and rounded apoptotic bodies as well as vacuole formation and pyknosis. This proves the process of apoptosis.

This finding suggests that the reduction observed in the viable cells following treatment with *Aaptos suberitoides* extract is due to cell death.

## CONCLUSION

From this study, it can be concluded that the extract of *Aaptos suberitoides* has a cytotoxic effect on triple negative breast cancer cell line. However, this research is still limited because it only uses simple in vitro testing to determine the ability of cytotoxicity.

All data in this study support that *Aaptos suberitoides* marine sponge has prospect for further research, with the result that it could become a complementary therapy in TNBC's standard treatment.

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