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Antioxidant and Cytotoxic Effect of Water Extract of *Ananas comosus* in Human Breast Cancer Cell Line

Rahma Micho Widyanto[1]*, Ririt Nafi' Halimah[1], Yosfi Rahmi[1], Budi Utomo[2], Wahyu Diah Proborini[3], Yunimar[4]

[1]School of Nutrition, Faculty of Medicine, Universitas Brawijaya Jl. Veteran, Malang 65145
[2]Faculty of Animal Husbandry, Universitas Nusantara PGRI Kediri Jl. KH Achmad Dahlan Mojoroto Kediri, 64112
[3]Chemical Engineering Department, Faculty of Engineering, Universitas Tribhuwana Tunggadewi Jl. Telaga Warna Tlogomas Malang, 65144
[4]Research Institute for Citrus and Subtropical Horticulture Crops. Jl. Raya Tlekung No.1, Junrejo, Kota Batu, 63105

Keyword :
Ananas comosus;
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A b s t r a c t

Background: *Breast cancer is the most common type of cancer and the second leading cause of death in the world. One of the supporting therapeutic efforts to overcome cancer is through food, mainly fruits. Ananas comosus has been investigated for its potential as an anti-cancer. It is a source of antioxidants from the content of Vitamin C and flavonoids that work by capturing free radicals, resulting in inhibition of cancer cell proliferation.* ***Objective :****This study was conducted to determine the antioxidant activity and cytotoxicity of water extract of Ananas comosus on T-47D breast cancer cell lines.* ***Method:*** *The process of extracting pineapple flesh is done using water as a solvent, then tested its antioxidant activity using DPPH and cytotoxicity using MTT assay on T-47D.* ***Result****: IC_{50} value for antioxidant activity was 463.369 µg / mL and the IC_{50} for the cytotoxicity assay was 488.003 µg / mL.* ***Conclusion*** *: The water extract of Ananas comosus have very weak antioxidant activity and moderate cytotoxicity properties that have potential as chemo preventive agents in T-47D breast cancer cell lines.*

**Corresponding author*
Email: micho@ub.ac.id

Introduction

Breast cancer is the main type of cancer found and the second leading cause of death in the world. This disease causes the death of sufferers, especially in women aged 45-55 years [1]. The incidence of breast cancer in the world occurs to 1 in 8 women that require tissue removal treatment, chemotherapy, radiotherapy, and hormone therapy [2] . While data onto Indonesia recorded 1 out of 2000 women suffering from breast cancer [3].

The most popular treatment for cancer is radiotherapy and chemotherapy. This combination is considered the most effective in healing cancer. However, some disadvantages of this method include radiotherapy that causes burns and damages healthy cells and tissues in the body. While chemotherapy can damage organs such as kidneys, liver and lungs [4]

One of the supporting therapeutic efforts to overcome cancer is through food, mainly fruits. There is a relationship between fruit consumption of a reduced risk of breast cancer [5]. Pineapple *Ananas comosus* (*A. comosus*) has been investigated for its potential as an anti-cancer. *A. comosus* is a source of antioxidants from the content of Vitamin C and flavonoids that work by capturing free radicals, resulting in inhibition of cancer cell proliferation [6 -9] . Compounds in *A. comosus* are known to inhibit the proliferation of cancer cells in leukemia, breast cancer, lung cancer, and cervix cancer [10].

In our previous study, the IC_{50} value of the methanol extract of pineapple showed antioxidant activity was 1549.88 µg/ml and cytotoxicity assay using T- 47D breast cancer cell line was 741.46 µg/ml, respectively [11]. This study aims to evaluate the potential antioxidant and anti-cancer activity of *A. comosus* extract. The choice of water solvent is due to the fact that pineapple processing is most often found in the form of juice with a mixture of pineapple flesh and water. In addition, water is a solvent that is easy to find and inexpensive, stable, non-toxic and non-flammable [12]. Measurement of antioxidant activity using the DPPH method and anti-cancer assay using the *in vitro* MTT assay method of T-47D breast cancer cell line.

Research Method

In this study, we performed *in vitro* true experimental laboratory design. Antioxidant activity assay used 4 series of triplicate concentration with vitamin C as a positive control. Cytotoxicity assay applied post-test only control group design on 4 series of triplicate concentration with Cisplatin as a positive control.

Preparation of extract

Sample of *A. comosus* fruit used part of whole fruit flesh with crown, weighing at least 300 g, the skin color of the fruit starts to turn yellow, clean and free from foreign scents. After that, the extraction is carried out using water as a solvent by the maceration method. The water extract was then tested for antioxidant activity using the DPPH method and cytotoxicity test using MTT assay with a concentration range of 125 ppm, 250 ppm, 500 ppm, and 1000 ppm. Controls for antioxidant and cytotoxicity testing were ascorbic acid and cisplatin, respectively. The test results will be analyzed to determine the IC_{50} value.

A. comosus was obtained from Pujon traditional market, Malang East Java.The making of *A. comosus* water extract was based on a modified method. A total of 150 g of pineapple flesh is divided into 3 and 200 ml of water is added respectively, soaked for 40 minutes. After 40 minutes the results of the immersion were heated by the water bath method for two hours with a temperature range of 70-80 ° C. Then the heating results were separated between the liquid and the solid by centrifugation at a speed of 10,000 rpm for 15 minutes. The supernatant is then evaporated by the water

bath method in the range of 90-100 ° C for 7 hours until the liquid becomes concentrated and leaves little water content. After evaporating, a vacuum oven process is carried out until pineapple fruit water extracts are obtained [13].

Cell culture

Culturing T-47D Breast Cancer Cell

T-47D cell was obtained from BPPT LAPTIAB Cell Culture Laboratory Puspiptek Serpong, at 6^{th} passage. Culturing of T-47D was conducted using RPMI 1640 and 10% fetal bovine serum. The T-47D cells were thawed with gentle stirring on water bath with a temperature of 37° C for 2 minutes. After thawing, the T-47D cells were removed and decontaminated using 70% ethanol under strict aseptic conditions. Subsequently, T-47D cells were transferred to a centrifuge tube containing 9.0 mL of complete culture medium, then centrifuged about 125 x g for 5-7 minutes. The supernatant resulted from centrifugation was discarded and the cells were dissolved in new media. T-47D cells were incubated at 37° C in incubator with 5% CO_2 and cultured for 3-7 days untuk reach 80% confluency.

DPPH Free Radical Scavenging Activity Assay

Antioxidant activity of *A. comosus* water extracts was analyzed using modification methods [14;16]. As much as 2 mg DPPH was dissolved using 100 ml of methanol in a 100 mL volumetric flask. Furthermore, sample solution of *A. comosus* fruit water extract concentration of 1500 µg/mL was prepared. The variation of the concentration is made form stock to concentration of 125; 250; 500; 1000 µg/mL. Measurement of antioxidant activity done by taking 6 ml of a standard solution of various concentrations and adding 4 ml of each DPPH solution, homogenized and incubated at dark room temperature for 30 minutes. 5 - 10 minutes before the incubation ends, each solution homogenized using vortex for 10 seconds. Results were read using a UV-Vis spectrophotometer at a wavelength of 516 nm. Ascorbic acid and methanol were used as control.

Cytotoxic assay

The cytotoxic assay in this study was a modification method [17]. The assay conducted by preparing 96-well plates and then plating T-47D cells in 100 ml of RPMI medium with $5x10^4$ cells/wells. Each time filling 12 wells, resuspension of cells to remain homogeneous. Cells are incubated in a CO_2 incubator overnight so the cells recover after harvesting.

Cell treatment was done by turning the plate in an appropriate container, then the remaining liquid is drained with a tissue. Cells were washed using 100 µL PBS 1x before being treated with a series triplicate concentration in the range of 125; 250; 500; 1,000 ppm. Cells were incubated in a CO_2 incubator for 24 hours. Positive control using cisplatin.

MTT [Sigma-Aldrich] was done by removing the culture media containing the test compound, then washed with 100 µL PBS 1x. 100 µL MTT was added to the culture media (0.5 mg/ml) each well. The plate is incubated in a CO_2 incubator for 3-4 hours. MTT reaction was stopped by adding 10% SDS in 0.01N HCl to the media containing MTT of 100 µL each well, then incubated overnight at room temperature with aluminum foil covered and darkroom conditions. The plate is shaken to a 100rpm shaker for 10 minutes to dissolve formazan. Living cells will react with MTT and form a purple color. The absorbance results are read using an ELISA reader at a wavelength of 570 nm.

Data analysis to determine IC_{50} values using Microsoft Excel 2010 and IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, NY, USA) SPSS [15 – 16].

Results and Discussions

Water extract of A. comosus

14 g of *A. comosus* fruit water extract was generated by extracting 150 g of fruit meat and produced 9.33% of yield. Yield is influenced by temperature where the optimum temperature will produce maximum yield [17]

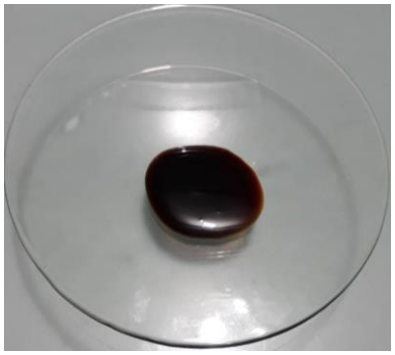

Figure 1. Water extract of *A. comosus*

Antioxidant activty

The highest free radical inhibition results obtained by 63.68 % at a concentration of 1000 µg/ml of *A. comosus* water extract (Table 1), with IC_{50} values of 463.369 µg/ml (Table 2). Vitamin C used as a positive control with the highest inhibitory value at a concentration of 2 µg/ml at 90.636% (Table 1) and the IC_{50} value of 1.17 µg/ml.

The purpose of antioxidant activity assay of *A. comosus* water extract by using the DPPH method is to find out the capacity of extracts to prevent free radicals. Antioxidant properties based on the IC_{50} value are divided into 4, which is very strong if the IC_{50} value <50 µg/mL, strong if the IC_{50} value is 50-100 µg/mL, moderate if the IC_{50} value is 101-150 µg/mL, weak if the IC_{50} value is 150- 200 µg/mL, and is very weak if IC_{50} value is > 200 µg/mL [18]. The IC_{50} results in this study were 463,369 µg/ml and the antioxidant activity were classified as very weak.

Table 1. DPPH activity of Water Extract of ***A.*** *comosus* and Water Vitamin C

Sample	**Concentration (µg/ml)**						
	0.5	1	2	125	250	500	1000
Ascorbic acid	14.606 ± 2.247[a]	44.943 ± 18.700[bc]	90.636 ± 1.299[d]	-	-	-	-
***A.comosus* extract**	-	-	-	15.964 ± 4.890[a]	37.543 ± 1.691[b]	57.368 ± 1.897[bc]	63.684[c]

Data were presented as mean ± standard deviation. Different letter in concentrations of samples indicate significant differences (Tukey's HSD post hoc test). Each sample was performed in triplicate

Table 2. IC_{50} Value of Antioxidant Activity using DPPH

Sample	**Linier Equation**	**R^2**	**IC_{50} (µg/ml)**
Water Extract of *A. comosus*	y = 54.141x - 94.336	0.95	463.369
Vitamin C	y = 49.97x – 8.24	0.99	1.17

A. comosus contains 24mg/100g vitamin C which is an antioxidant agent [19]. Vitamin C is a water-soluble vitamin that can be extracted from water solvents. However, vitamin C is very susceptible to high temperatures. In this study extraction was carried out using conventional extraction methods with temperatures ranging from 60°C-100°C. This result confirmed with other research that levels of vitamin C from tomato pasta continue to decrease ranging from 40°C, 60°C and 80°C with heating for 200 minutes [20] . In Passion fruit, the content of vitamin C if stored at room temperature (23°C) for 5 hours will reduce vitamin C levels by as much as 6.3% [21]. It can be concluded that the treatment of high temperatures in vitamin C will cause the molecular constituents of

vitamin C to be broken so that vitamin C becomes decomposed and damaged.

In this research, the solvent used for extracting *A. comosus* are water. It was found that the ability of the free radical from *Annosa squamosa* extract for each solvent from the largest to the smallest was acetone (70.92%) > methanol (47.62%) > ethanol (26.00%) > water (7.27%) [22]. Antioxidant of *Annona muricata* Linn. juice also shown very weak activity 3549 µg/ml [23]. Based on these studies it is suspected that water solvents are less able to attract active substances than other solvents, so the antioxidant activity of the extract is very weak.

Cytotoxic activity

The highest inhibition proliferation of *A. comosus* water extract against T-47D breast cancer cell lines were 58.773 % at a concentration of 500 µg/ml (Table 3), with IC_{50} values of 488.003 µg/ml (Table 4). Cisplatin used as a positive control with the highest inhibitory value at a concentration of 12 µg/ml at 73.186 % (Table 4) and the IC_{50} value of 8.64 µg/ml. The level of cytotoxicity was divided into 3 groups, whereas potential cytotoxicity if IC_{50} <100 µg/mL, moderate cytotoxicity if IC_{50} was 100 - 1000 µg/mL, and non-toxic if > 1000 µg/mL. In the group of compounds with potential cytotoxicity, it could be useful as an anti-cancer, whereas in compounds with moderate cytotoxicity can function as chemo prevention agent, which can only prevent and inhibit the growth of cancer cells [24]. The results of this study indicate that the cytotoxicity of *A. comosus* water extracts is moderate against T-47D breast cancer cell lines.

Table 3. Cytotoxicity of Water Extract of *A. comosus* in T-47D cell line

Sample	**Concentration (µg/ml)**							
	3	6	9	12	62.5	125	250	500
A.comosus extract	-	-	-	-	24.497 ± 1.371[b]	38.503 ± 3.825[c]	49.834 ± 0.345[d]	58.773 ± 1.260[e]
Cisplatin	9.778 ± 3.706[a]	23.097 ± 1.113[b]	60.337 ± 0.955[e]	73.186 ± 0.229[f]	-	-	-	-

Data were presented as mean ± standard deviation. Different letter in concentrations of samples indicate significant differences (Tukey's HSD post hoc test). Each sample was performed in triplicate

Table 4. IC_{50} Value of Cytotoxicity Assay using MTT

Sample	**Linier Equation**	**R^2**	**IC_{50} (µg/ml)**
Water Extract of *A. comosus*	y = 0.0616 + 19.939	0.95	488.003
Cisplatin	y = 7.5834x – 15.266	0.96	8.64

In this study crude extracts used as samples, whereas the content of compounds that have cytotoxic properties is still mixed with other compounds. In previous studies showed that the IC_{50} of cytotoxic assay of *Pandanus conoideus* Lamk. methanol extracts were 352.72 µg/ml and classified as moderate cytotoxic properties because the extraction produced was crude extract [25]. Therefore, A. comosus water extract requires furthrt analysis to determine which substance act as cytotoxic agents [26].

Pineapple has been tested for its cytotoxic activity against several cancer cells. The methanol extract of pineapple can inhibit the growth of HepG2 liver cancer cells with IC_{50} values of 22.4 µg / ml [27]. In addition, pineapple fruit juice also has cytotoxic properties that are more active in HT29 colon cancer cells with IC_{50} values of 104.95 µg / ml. Our findings demonstrated that pineapple extract exhibited the lowest cytotoxic activity, this

shows that T-47D breast cancer cells are less sensitive to pineapple juice extracts [28].

From the results of the antioxidant and cytotoxic assays have almost the same results. One of the pineapple's content is vitamin C. The Vitamin C is water soluble, so it is possible that the substance can be extracted by water [29]. Aside from being an antioxidant, vitamin C also has an important role in dealing with anti-cancer properties. In studies of MCF7 and HT29 cancer cells, it is thought that vitamin C can inhibit the growth of cancer cells by inhibiting the formation of nicotinamide adenine dinucleotide (NAD). NAD is a substance that is needed for the process of glycolysis, if this process continues not to occur it will result in death of cancer cells. In addition to these mechanisms, vitamin C inhibits cancer growth by inducing the formation of hydrogen peroxide in tissues that act as cytotoxic agents in cancer cells [30].

Conclusions

It can be concluded that *A. comosus* water extracts have very weak antioxidant activity and moderate cytotoxicity properties that have potential as chemo preventive agents in *in vitro* T-47D breast cancer cell lines.

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