Studies on the Antioxidant and Cytotoxic Potentials of the Peel Extract of Dacryodes rostrata

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Abstract: Breast cancer is one type of cancer that causes the highest death in women in Indonesia. Alternative herbal-based cancer treatments have been developed, one of which is using fruits. Dacryodes rostrata, a fruit commonly consumed by residents in Kalimantan, is rich in antioxidants such as flavonoids and phenolics. The purpose of this study was to determine the value of antioxidant and cytotoxic activity of water and ethanol extract of D. rostrata peel against T-47D breast cancer cell lines. The fruit extraction was carried out by using maceration method. Antioxidant activity test using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and cytotoxic test using MTT method (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenitetrazolium bromide) assay. The results showed that D. rostrata water and ethanol extract had antioxidant activity with IC_{50} values 121.7 ppm and 59.27 ppm, respectively. While cytotoxic effect on T-47D cells with IC_{50} values of 322.55 ppm and 143.02 ppm, respectively. This study showed that D. rostrata peel water extract had moderate antioxidant activity and moderate cytotoxic effect against T-47D breast cancer cells in vitro which could be used as a chemo preventive to prevent and inhibit cancer cell growth.
Keywords: Dacryodes rostrata, Antioxidant, Cytotoxic, Breast cancer, T-47D cell line

1 Introduction

Breast cancer is a condition of the emergence of malignant tumours originating from the breast glands. In breast cancer, there is an abnormal proliferation of cells until the cells get out of control and form a lump in the breast. Breast cancer is one type of cancer that causes the highest death in women in Indonesia. Breast cancer in Indonesia was ranked first in the incidence of new cases and ranked second in deaths from cancer where the prevalence was highest among females (30.9%) [1].

Cancer treatment efforts can be done through radiation, surgery, chemotherapy, and hormone therapy. Among these efforts, the therapy that is considered the most effective for breast cancer is chemotherapy. However, this therapy has side effects that do not only kill cancer cells but also kill normal cells in the body, causing nausea and vomiting, and also trigger cachexia [2]. To overcome these problems, cancer treatment can also be pursued with herbal treatment, a treatment carried out by utilizing materials from plants [3].

D. rostrata is one of Indonesia's endemic fruits originating from Kalimantan and can be found in all districts in West Kalimantan. This fruit is usually consumed by soaking it in hot water for a few minutes until the flesh is edible. The flesh and skin of *D. rostrata* fruit can be an important source of energy and minerals for humans. In addition, *D. rostrata* has a rich content of protein, minerals, and oils. *D. rostrata* also has antioxidant compounds such as flavonoids and phenols that can prevent oxidative stress and can work against cancer through the mechanism of activating the apoptotic pathway of cancer cells [4, 5, 6, 7].

This study aims to evaluate the potential antioxidant and anti-cancer activity of *D. rostrata* extract. Measurement of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and anti-cancer assay using the *in vitro* 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay method of T-47D breast cancer cell line.

2 Research Method

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2.1 Preparation of extract

The fruit of *D. rostrata* purchased in Malawi Regency, West Kalimantan. The sample used is peel flesh and extracted with water and ethanol as a solvent. The extract was then tested for antioxidant activity using the DPPH method with concentration range from 62.5; 125; 250; and 500 ppm. And cytotoxic assay using MTT with concentration range from 62.5; 125; 500; and 1000 ppm. Controls for antioxidant and cytotoxic assay were ascorbic acid and cisplatin, respectively. The test results will be analyzed to determine the IC_{50} value.

The making of *D. rostrata* water and ethanol 50% extract was based on a modified method [8]. A total of 50 g peel flesh dissolved into 250 ml of water or ethanol 50%. For water extraction, 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 10000 rpm for 15 minutes. For ethanol 50% extraction, sample were separated using centrifuge at 1000 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. After evaporating, a vacuum oven process is carried out until peel extracts are obtained.

2.2 Antioxidant Activity

Antioxidant activity assay was based on modified method [9]. 50 ppm DPPH solution [Sigma-Aldrich] was prepared by weighing 5 mg of DPPH dissolved with 100 mL of methanol in a volumetric flask. The stock solution of *D. rostrata* peel water extract was made at 1500 ppm by weighing 150 mg of *D. rostrata* peel and dissolved with 1 mL DMSO [Merck] and methanol to 100 mL. From the stock solution, the test solution was made into 4 concentrations (62.5; 125; 250; 500 ppm). The comparison solutions were also made. A stock solution of 100 ppm was prepared by weighing 1 mg of ascorbic acid and diluted with methanol until it was homogeneous and the volume was made up to 10 mL. Concentration variations were made to 0, 5, 1, and 2 ppm.

D. rostrata peel water extract and ethanol 50% extract of *D. rostrata* peel and ascorbic acid were taken 6 mL from each concentration and on each were added 4 mL of DPPH. The solution was then vortexed and incubated in the darkroom at 37°C. Absorbance measurements were carried out at a wavelength of 517 nm. The antioxidant activity was conducted using 4 series of triplicate concentration with ascorbic acid as a positive control.

2.3 Cell Culture and Cytotoxic Assay

Cell culture and cytotoxic assay were based on modified method [10]. T-47D breast cancer cells were obtained from the Cell Culture Laboratory of BPPT-LAPTIAB Puspiptek Serpong. T-47D breast cancer cells were thawed with gentle stirring on an air bath at 37°C for 2 minutes. After the dilution, T-47D breast cancer cells were removed and decontaminated using 70% ethanol under strict aseptic conditions. The T-47D breast cancer cells were then transferred to a centrifuge tube containing 9 mL of complete culture medium, then centrifuged [Hettich] about 125 x g for 5-7 minutes. The centrifuged supernatant was discarded and the cells were dissolved in the new medium. Subsequently, T-47D breast cancer cells were incubated at 37°C in a 5% CO_2 incubator [Memmert] and cultured for 3 – 7 days to achieve 80% confluency.

The cells ($5x10^4$ cells/100 µl) were regenerated, then distributed into wells on 96-well plates, the homogeneity of cell suspensions was maintained by resuspension them periodically, while three wells were left for media control. The cells were incubated in a CO_2 incubator overnight.

The cell medium was removed with a 180° plate. Each well was filled with 100 PBS and discharged again with a 180° plate. The concentration series of water extract of *D. rostrata* peel and ethanol 50% extract of *D. rostrata* peel (62.5; 250; 500; 1000 ppm) and cisplatin (3; 6; 9; 12 ppm) were inserted into the well (triplicate). The cells were then incubated in a CO_2 incubator for 24 hours.

100 µl MTT [Promega] was added to the RPMI culture medium [Gibco] (0.5 mg/ml) and then incubated in a CO_2 incubator for 3-4 hours. The cell conditions were examined using an inverted microscope. The MTT reaction was stopped when formazan was formed clearly by adding 100 l stopper in 0.1 N HCl in the medium containing MTT. The incubation was carried out at room temperature in the dark. After incubation, the plates were shaken on a shaker. Then the absorbance was measured at a wavelength of 570 nm using an ELISA reader [BioTek]. The cytotoxic assay was applied post-test only control group design on 4 series of triplicate concentrations with Cisplatin as a positive control.

2.4 Data Analysis

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.

3 Results and Discussions

3.1 Antioxidant activity

Tests of antioxidant activity on water extract of *D. rostrata* peel, ethanol 50% extract of *D. rostrata* peel, and ascorbic acid as a comparison with the DPPH method showed changes in each concentration. The results of absorbance and percent inhibition shown in Table 1. are the average of the 3 replications that have been carried out.

Antioxidant activity testing is used to measure the ability of antioxidants to reduce free radicals. The results of the antioxidant activity test using ethanol 50% extract and water extract of *D. rostrata* peel have antioxidant activity with IC_{50} values of 121.7 ppm and 59.27 ppm respectively. The strength of antioxidant activity is divided into 5 categories, namely very strong if the IC_{50} value is <50 ppm, strong if the IC_{50} value is 50-100 ppm, moderate if the IC_{50} value is 101-250 ppm, weak if the IC_{50} value is 251-500 ppm, and inactive if the IC_{50} value is >500 ppm [11]. Based on these categories, the antioxidant activity of the ethanol 50% extract of *D. rostrata* peel can be categorized as moderate, while the antioxidant activity of

the water extract of *D. rostrata* peel is categorized as strong antioxidant activity.

Several factors can affect the decrease in antioxidant activity, one of which is the heating process when making extracts. Phenol compounds can undergo changes caused by the heating process [12]. The higher the temperature used during the heating process, the phenolic compounds that act as antioxidants decreased, hence the strength of antioxidant activity decreased as well [13]. A long heating process with high temperatures can cause a decrease in antioxidant activity [14]. In this study, water extract and ethanol 50% extract of *D. rostrata* peel were heated using an oven and a vacuum oven to remove solvents. The existence of the heating process is thought to be one of the factors that cause a decrease in the ability of phenolic and flavonoid compounds that function as free radical scavengers, resulting in a decrease in the strength of antioxidant activity.

Another factor that affects the antioxidant activity in this study is the solvent used. Different solvents can affect the IC_{50} antioxidant activity results. A very significant difference in the solvent used in the same sample can result in a higher or lower IC_{50} [15]. The results of the research on the antioxidant activity of water extract and ethanol 50% extract of *D. rostrata* peel showed that water solvent produced higher antioxidant activity. This result is in line with the previous research that the antioxidant activity of *D. rostrata* peel pulp that is produced from various solvents such as hexane, ethyl acetate, butanol, ethanol 50%, and water, had different results [16]. In this study, it was seen that water produced a higher antioxidant activity than ethanol 50%. The effect of the type of solvent on the samples tested resulted in different results. This is due to the different nature of the polarity of the bioactive compounds of each fruit so that the solvent with similar polarity to the fruit being tested will attract a lot of the bioactive components present in the fruit.

Table 1. DPPH activity of Extract of *D. rostrata*

Sample	Concentration (ppm)						
	0,5	1	2	62,5	125	250	500
Ascorbic acid	14.61 ± 2.47	44.95 ± 18.7	90.64 ± 1.3	-	-	-	-
Water Extract	-	-	-	49.33 ± 0.53	68.33 ± 0.41	91 ± 4.16	98.33 ± 0.27
Ethanol 50% Extract	-	-	-	27.6 ± 0.65	47.98 ± 1.04	78.87 ± 2.63	97.13 ± 0.26

Data were presented as mean ± standard deviation. Each sample was performed in triplicate

3.2 Cytotoxic activity

In the cytotoxicity test, the MTT method was used using cisplatin as a positive control. The absorbance used to calculate the percent inhibition of proliferation in T-47D cancer cells which can be seen in Table 2 is the average of the 3 replications that have been carried out.

In this test, water extract and ethanol 50% extract of *D. rostrata* peel respectively produced IC_{50} of 322.55 ppm and 143.02 ppm. If the value is 100 ppm < IC_{50} < 1000 ppm, the strength can be categorized as moderate cytotoxic, hence the second extract has a cytotoxic effect and is included in the moderate category [17]. Based on this classification, it is known that the ethanol 50% extract of *D. rostrata* peel is moderately cytotoxic.

In a previous study regarding the antioxidant content found in the flesh of the *D. rostrata* peel air solvents and ethanol 50%, it shows that the highest antioxidant content comes from phenols and flavonoids [16]. Flavonoids can activate p53 protein by inhibiting tumor growth through cell cycle and apoptosis [18]. Meanwhile, the content of phenolic compounds can increase the inhibition of cancer cells [19]

Table 2. Cytotoxic of Extract of *D. rostrata* in T-47D cell line

Sample	Concentration (ppm)							
	3	6	9	12	62,5	125	250	500
Cisplatin	9.78 ± 3.71	23.1 ± 1.11	60.38 ± 0.96	73.19 ± 0.23	-	-	-	-
Water Extract	-	-	-	-	40.3 ± 0.67	54.34 ± 0.04	52.15 ± 1.93	68.62 ± 0.63
Ethanol 50% Extract	-	-	-	-	48.2 ± 0.21	49.53 ± 0.35	58.53 ± 0.11	71.42 ± 1.12

Data were presented as mean ± standard deviation. Each sample was performed in triplicate

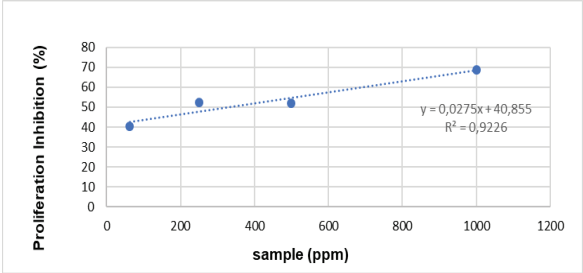

Fig. 1. Linear Regression Curve of Water Extract of *D. rostrata* Concentration with Percentage of Inhibition of T-47D Cell Proliferation.

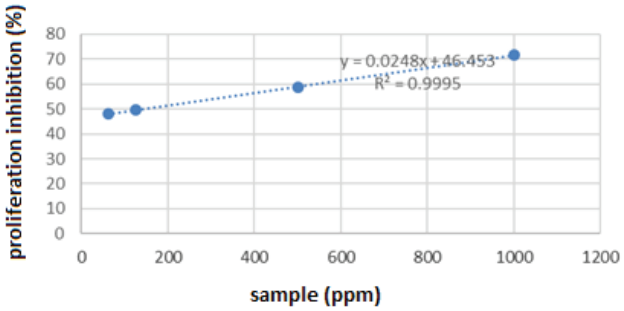

Fig. 2. Linear Regression Curve of Ethanol 50% Extract of *D. rostrata* Concentration with Percentage of Inhibition of T-47D Cell Proliferation.

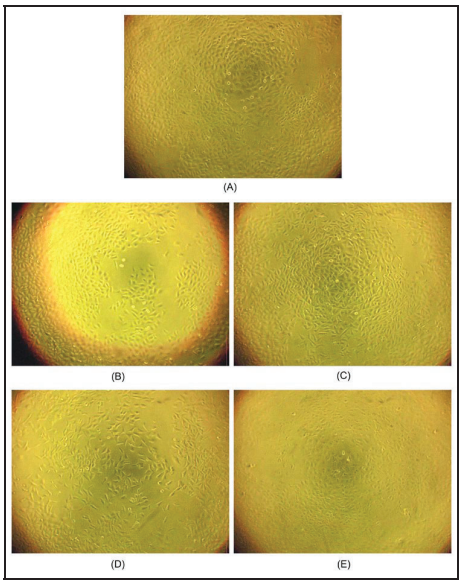

Fig. 3. T-47 D Cell line Treated with Water Extract of *D. rostrata.* (A) 0 ppm; (B) 62.5 ppm; (C) 125 ppm; (D) 250 ppm; (E) 500 ppm.

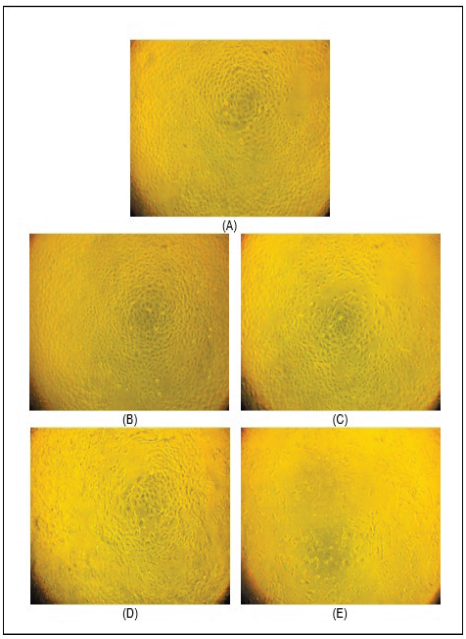

Fig. 4. T-47 D Cell line Treated with Ethanol 50% Extract of *D. rostrata.* (A) 0 ppm; (B) 62.5 ppm; (C) 125 ppm; (D) 250 ppm; (E) 500 ppm.

4 Conclusions

It can be concluded that the water and ethanol 50% extract of *D. rostrata* peel had moderate antioxidant activity and cytotoxic effects on T-47D breast cancer cells in vitro so that they can be used as chemo preventives to prevent and inhibit the growth of cancer cells.

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References

1. F. Bray, et al., Global Cancer Statistic 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 countries, C. Cancer J Clin, **68** (2018).
2. AC. Wijaya and M. Muchtaridi. Pengobatan Kanker Melalui Metode Gen Terapi, Farmaka, **15,** 1 (2017).
3. Parwata, IMDOA. Obat Tradisional. Laboratorium Kimia Organik Fakultas Matematika dan IPA Universitas Udayana. Bali (2016).
4. SDS. Banjarnahor and N. Artanti, Antioxidant Properties of Flavonoids, Med. J. Indonesia, **23**, 4 (2014).
5. A. Monasterio, et al, Flavonoids Induce Apoptosis in Human Leukemia U937 Cells Through Caspase- and Caspase-Calpain-Dependent Pathways, Nutr and Canc, 2004, **50**, 1 (2004)
6. K. Mulia, et al. Total Phenolic, Anticancer and Antioxidant Activity of Ethanol Extract of Piper retrofractum Vahl from Pamekasan and Karang Asem, Curr Biochm, **3**, 2 (2016).
7. K. Sak and H. Everaus. Multi-Target Cytotoxix Actions of Flavonoids in Blood Cancer Cells, Asian Pac J. of Canc Prev, **16**, (2016).
8. J. Suomi, et al. Extraction of iridoid glycosides and their determination by micellar electrokinetic capillary chromatography. J. of Chrom. A. **868**, 1 (2000)
9. W. Widowati. et al, Phytochemical, Free Radical Scavenging and Cytotoxic Assay of Cucumis Melo L. Extrand and β-Carotene, J. of Adv Agri Tech, **2**, 2 (2015).
10. RM. Widyanto, et al. Antioxidant and Cytotoxic Effect of Water Extract of Ananas comosus in Human Breast Cancer Cell Line. J. of Islamic Medicine, **4,** 2 (2020).
11. JKSL. Lung and DP. Destiani, Uji Aktivitas Antioksidan Vitamin A, C, E dengan Metode DPPH, Farmaka, **15**, 1 (2017)
12. CY. Park, et al. Phenolics and Antioxidant Activity of Aqueous Turmeric Extracts as Affeceted by Heating Temperature Time, Food Sci and Tech,**105** (2019).
13. RB. Pertiwi, et al. Aktivitas Antioksidan Ekstrak Buah Parijoto pada Berbagai Suhu Pengolahan Pangan, J. Ilmu Pangan dan Hasil Pertanian, **3** 1 (2019).
14. A. Husni, et al., Aktivitas Antioksidan Padina sp. Pada Berbagai Suhu dan Lama Pengeringan, JPB Perikanan, **9,** 2, (2014).
15. H. Truong, et al. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-

Inflammatory Activities of Severinia buxifolia. J. of Food Quality (2019).
16. N. Thavamoney, et al. Extraction and Recovery of Phytochemical Components and Antioxidative Properties in Fruit Parts of Dacryodes rostrate Influenced by Different Solvents, J Food Sci Technol, (2018)
17. P. Prayong, S. Barusrux and N. Weerapreeyakul. Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia, **79** (2008)
18. B. Plaumann, et al. Flavonoid Activate Wild-Type p53, Oncogene, **13**, 8 (1996).
19. A. Tsiapara, et al. Bioactivity of Greek honey extracts on breast cancer (MCF7), prostate cancer (PC3) and endometrial cancer (Ishikawa) cells: Profile analysis of extracts. Food Chemistry – Food Chem. **116** (2009).