

Antioxidant and Anticancer Potential of Raja Bulu Banana Peel and Blossom (*Musa acuminata* Colla (AAB group)) Ethanol Extracts in MCF-7 Cell Lines

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ABSTRACT

Breast cancer is an uncontrolled cell growth in breast tissue. Surgical treatments of breast cancer can reduce breast aesthetics and chemotherapy can cause severe side effects. It makes the searches for plants as breast anticancer agents intensively carried out. Several studies have shown that banana peels and blossoms possess antioxidant and anticancer activity. This study aims to determine the fruit peel and blossom of Raja Bulu banana (*Musa acuminata* Colla (AAB group)), an endemic banana species in Indonesia, potential as antioxidant and anticancer agent in MCF-7 cells. Antioxidant potential was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H₂O₂) scavenging activity assay. Anticancer potential was determined by cytotoxic test using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). The results showed that 70% ethanol extract of fruit peel (PBEE) and blossom (BBEE) of Raja Bulu banana had median inhibition concentration (IC₅₀) for DPPH scavenging activity at 115.32 µg/mL and 162.52 µg/mL respectively, while for H₂O₂ scavenging activity at 624.80 µg/mL and 497.13 µg/mL respectively. Anticancer potential was expressed by inhibiting concentration of 50% proliferation (IC₅₀) of MCF-7 cells for PBEE and BBEE were 115.00 µg/mL and 338.47 µg/mL respectively. This study showed that PBEE and BBEE have antioxidant and anticancer properties.

Key words: Anticancer; Antioxidant; *Musa acuminata*; MCF-7

INTRODUCTION

Breast cancer is a malignancy in breast epithelial tissue that can originate from the breast ducts (lactiferous ductus) and breast glands (lobules) (National Cancer Institute, 2009). Breast cancer is a disease with high mortality and incidence rate in the world (Houghton & Chamberlain's, 2012). Besides cancer risk could be increased by post-translational protein modifications or mutations in cancer-related genes by some reaction like nitration, nitrosation, phosphorylation, acetylation, and poly (ADP-ribose) ation. It occurs as the result of free radicals or lipid peroxidation byproducts presence such as malondialdehyde (MDA) and 4-hydroxynonenal (4HNE). Free radicals in carcinogenesis can damage DNA and modify the proteins structure and function that maintain cellular integrity and promote angiogenesis. The DNA damage by free radicals induces chromosome fragmentation. It has been demonstrated using hydrogen peroxide (H₂O₂). It means, free radicals can increase tumorigenesis through some way like DNA damage

and mutation, stimulating cell cycle/proliferation, inhibiting apoptosis, and inhibiting DNA repair (Widowati *et al.*, 2013a).

The lack of selectivity also the appearance of drug-resistant tumor are factors that limited the solid tumor chemotherapy. Surgery performed on early-stage breast tumors can reduce the aesthetics of the breast. Finding natural ingredients for chemotherapy agents continues to be the effort focus. Natural ingredients as cancer prevention is considered an approach to reduce the increasing cancer incidence (Ray *et al.*, 2010). Herbal medicine was containing antioxidants that could be the sources for replacing synthetic chemotherapeutic agents. It has advantages where they are less cytotoxic toward healthy cells thus safer to use (Widowati *et al.*, 2013a).

Various compounds produced by edible plants like flavonoids and polyphenols have shown anticancer activity. Flavonoids are a class of compounds that have been known to have antioxidant and anticancer activity. Polyphenols can inhibit carcinogenesis by influencing molecular pathways at the initiation, promotion, progression, and metastasis stages (Ferguson *et al.*, 2004).

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Based on previous research on antioxidant activity and anticancer screening of banana peels in n-hexane, ethanol, and water extracts against HUVEC, HCT-116, and MCF-7 cells was shown that the highest ability to inhibit MCF-7 cell growth was found in 12.07% ethanol extract of banana peels (Dahham *et al.*, 2016). The banana blossom is a food rich in nutrients that contains calcium, phosphorus, iron, and some vitamins A, B1, and C that beneficial for the human body (Sheng *et al.*, 2010). Therefore, the antioxidant and anticancer potential of 70% ethanol extract Raja Bulu banana (*Musa acuminata* Colla (AAB group)) peel and blossom in MCF-7 cells was determined in this study.

METHODOLOGY

Materials

Raja Bulu banana peel and blossom (*M. acuminata* Colla (AAB group)) were obtained from Perkebunan Pisang Raja Bulu in Jampang Kulon, Sukabumi, West Java and has been identified by Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The extract was obtained by using 70% ethanol and rotary vacuum evaporator (IKA RV 3 V-C) at 60°C. Phytochemical screening test was performed using 1% FeCl₃ (Merck, 1.03861.0250), acetate acid, absolute sulfuric acid (H₂SO₄) (Merck 109073), HCl 1 N (Merck 1003171000), vanillin (Merck 204-465-2), Mg powder (Merck EM105815), amyl alcohol (Merck 10979), also Dragendorff's reagent. The 2,2-diphenyl-1-picrylhydrazil (DPPH) assay was performed using DPPH (Sigma Aldrich D9132), DMSO (Merck 1029522500), microplate reader spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). Hydrogen peroxide (H₂O₂) scavenging assay was performed by using ferrous ammonium sulphate (1 mM, Sigma 7783859), H₂O₂ 5 mM (Merck 1.08597.1000), 1,10-phenanthroline 1 mM (Merck 200-629-2). Cytotoxic test was performed by using MTS testing (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (CellTiter 96® Aqueous One Solution Cell Proliferation Assay Promega G3581) and MCF-7 cell line (ATCC® HTB-22™).

Methods

Extract Preparation

The preparation of extract begins with the fresh Raja Bulu banana fruit peel and blossom collection was washed and chopped to be small pieces and weighed. It was oven-dried at 50°C. 300 g of Raja Bulu banana peel simplicia were macerated using 70% ethanol around 900 mL.

Meanwhile, 500 g of Raja Bulu banana blossom simplicia were macerated using 70% ethanol, filtered, and evaporated using rotary vacuum evaporator to produce a thick peel banana ethanolic extract (PBEE) and blossom banana ethanolic extract (BBEE) The extract was used for further testing and stored at -20°C (Walida *et al.*, 2016; Widowati *et al.*, 2017; Prahastuti *et al.*, 2020).

Phytochemical Screening Test

Phytochemical tests were carried out on PBEE and BBEE using the modified Farnsworth method to identify the presence of phenols, steroids/triterpenoids, saponins, terpenoids, flavonoids, and alkaloids qualitatively based on color reaction and precipitation that is typical of each secondary metabolite (Harbone, 1987; Widowati *et al.*, 2017; Widowati *et al.*, 2018; Prahastuti *et al.*, 2020). The method was listed below.

Phenols Identification

The sample (10 mg) was mixed with FeCl₃ in a dropping plate. The formation of green/red/blue/purple/black color was indicated phenols in the sample.

Steroid/Triterpenoids Identification

The sample (10 mg) was sinked in acetate acid at the dropping plate. Then, after 10 – 15 minutes, the mixture was mixed with H₂SO₄ as much as one drop. If green or blue color was presented that means the sample contain steroid, but if red or orange sediment was presented that means the sample contain triterpenoids.

Saponins Identification

The sample (10 mg) was inserted into the test tube that contain water. It was boiled in the water bath for 5 minutes. After that, it was shaken vigorously and dripped some HCl 1 N. If stable foam was present on the surface, it means the sample contain saponins.

Terpenoids Identification

The sample (10 mg), vanillin, also H₂SO₄ was mixed in the dropping plate. The terpenoids was present in sample if purple color was formed in the mixture.

Flavonoids Identification

The sample (10 mg) was mixed with Mg powder and HCl 2N in the test tube. Then it was heated for around 5 to 10 minutes. After that it was cooled and filtered to collect the filtrate. The filtrate was mixed was amyl alcohol. The red or

orange color formation was indicated flavonoids in sample.

Alkaloids Identification

The sample as much as 10 mg was dissolved in 5 mL of HCl 2 N. The mixture was separated into two test tube. The first tube was mixed with HCl 2 N as much as 3 drops and used as blank, while in the other tube the solution was placed in the dropping plate around 1 – 2 drops and used as sample. The sample was mixed with Dragendorff's reagent. If the color changes to orange it means the extract was positively containing alkaloids.

DPPH Scavenging Assay

DPPH assay was used to determine the DPPH scavenging activity of extracts. Briefly, 50 µL of PBEE and BBEE at various concentration and 200 µL of DPPH 0.077 mmol were added to 96 well plates as the sample well. The control was created using only DPPH 0.077 mmol as much as 250 µL, while the blank well was created using 200 µL of DMSO and 50 µL of PBEE and BBEE. The mixture was incubated in the dark at room temperature for 30 minutes. The microplate reader was used to analyze the absorbance at 517 nm. Each sample scavenging activity was determined by using formula below (Widowati *et al.*, 2017; Widowati *et al.*, 2018; Prahastuti *et al.*, 2020).

$$\text{scavenging\%} = \frac{Ac - As}{Ac} \times 100\%$$

Ac = Control absorbance; As = Samples absorbance
H₂O₂ Scavenging Assay

This assay was done by using Mukhopadhyay *et al.* (2016) method with modification. Briefly, ferrous ammonium sulphate as much as 12 µL, 60 µL of PBEE and BBEE at various concentration, and 3 µL of H₂O₂ 5 mM were added to 96 well plate as the sample well. The mixture was incubated at dark room temperature for 5 minutes. Briefly, 1,10-phenanthroline 1 mM as much as 75 µL was added into the mixture and incubated for 10 minutes at room temperature. The wavelength of 510 nm was used to determine the absorbance and it was used to measuring the scavenging activity by following equation (Utami *et al.*, 2017; Prahastuti *et al.*, 2020).

$$\text{H}_2\text{O}_2 \text{ Scavenging Activity (\%)} = \frac{As}{Ac} \times 100\%$$

Ac = Control absorbance (1,10-phenanthroline and ferrous ammonium sulphate); As = Samples absorbance

Cytotoxic Test

MTS test was used to determine the percentage of cell viability. The MCF-7 cells that

used in this study are continuous cell lines. The cells were planted with a density of 1×10^4 into a 96-well plate for 48 hours, then incubated until the cells reach 80% confluent. After 48 hours of incubation, the cells in each well were contacted with PBEE and BBEE, ~~epicatechin, and quercetin~~ with work solution at various concentrations in 10% DMSO and incubated for 24 h. Extracts that not added to the cell were used as blanks. Control cells was contained cells that not added the extracts. Whereas DMSO controls was contained cells added with 10% DMSO. MTS as much as 20 µL was added into each well and incubated for 3 hours at 37°C and 5% CO₂. The spectrophotometer was used to measuring the cell absorbance. The wavelength that used to measuring absorbance was 490 nm (Widowati *et al.*, 2013a; Widowati *et al.*, 2013b; Evacuasiyani *et al.*, 2014; Widowati *et al.*, 2015). The data were presented as percentage of living cells (%) and inhibition of cell proliferation (%). The following formula were used to calculating the percentage of living cells (%) and inhibition of cell proliferation (%). The Median Inhibitory Concentration 50 (IC₅₀) was determined using probit analysis.

$$\% \text{ cell viability} = (As - Ab) / Ac \times 100$$

$$\% \text{ inhibition of cell proliferation} = 100 - \% \text{ cell viability}$$

As = absorbance sample; Ab = absorbance blank;
 Ac = correction of the absorbance control average

RESULT AND DISCUSSION

The Raja Bulu banana peel and blossom have been reported to contain compounds that have potential as antioxidant and anticancer (Dahham *et al.*, 2015; Roobha *et al.*, 2011). The banana peel contains epicatechin while the banana blossom contains quercetin (Joseph *et al.*, 2014; Someya *et al.*, 2002).

Phytochemical screening of PBEE and BBEE were carried out to provide preliminary information regarding secondary metabolites that contained in the extract. The results of phytochemical screening can be seen in Table 1. It was shown that PBEE contained flavonoids, polyphenols, and terpenoids. The results was different with the phytochemical screening from previous studies on the Raja Bulu banana skin. Someya *et al.* (2002) confirms a presence of polyphenol compounds in the Raja Bulu banana skin (Someya *et al.*, 2002), while Guntarti *et al.*, (2015) shows a positive results for flavonoid, saponins, and the and negative results for alkaloids, steroids, and triterpenoids compounds.

Meanwhile, phytochemical screening results of Raja Bulu banana blossom extracts shown containing flavonoids, polyphenols, and

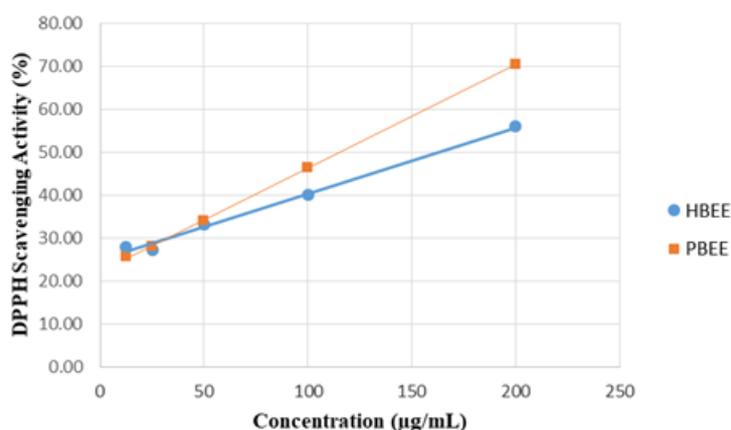


Figure 1. Antioxidant activity of PBEE and BBEE at various concentration against DPPH radical scavenging.

*The activity was presented as mean. The assay was done triplicate for each concentration and sample. Sample concentrations: 12.5 ug/mL; 25 µg/mL; 50 µg/mL; 100 µg/mL; and 200 µg/mL.

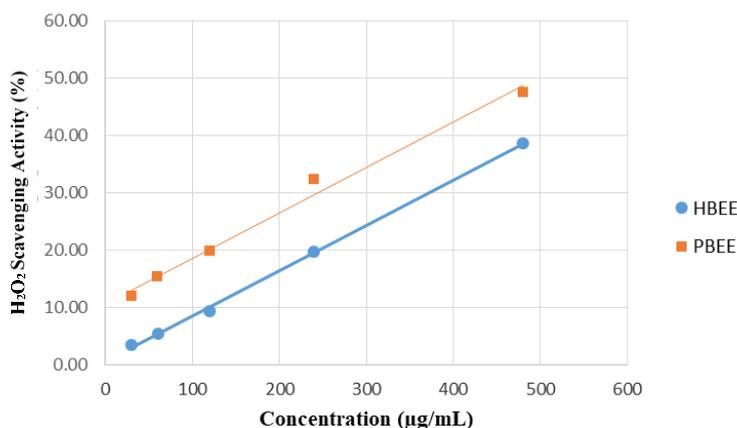


Figure 2. Antioxidant activity of PBEE and BBEE at various concentration against H₂O₂ radical scavenging.

*The activity was presented as mean. The assay was done triplicate for each concentration and sample. Sample concentrations: 30 µg/mL; 60 µg/mL; 120 µg/mL; 240 µg/mL; and 480 µg/mL.

terpenoids. This result was different with previous studies where in this study shown positive results on alkaloids compounds while the other previous research shown the presence of saponins compounds in the Raja Bulu banana blossom (Joseph *et al.*, 2014; Pane, 2013). The difference in phytochemical screening results is due to the content of secondary metabolites affected by the conditions of the growth place, physical factors (temperature, light, humidity, etc.), genetic factors, environmental stress factors (metal heavy, UV light), and type of solvent used (Mahmoo *et al.*, 2011).

DPPH and H₂O₂ scavenging activity were used to determine the antioxidant activity. Active antioxidant in DPPH free radical scavenging is marked with a color change from dark purple to

pale yellow or yellowish (Evacuasiyany *et al.*, 2014; Prahastuti *et al.*, 2020). While H₂O₂ scavenging activity was based on the ferrous ammonium sulphate and phenanthroline reaction that could form Fe²⁺-tri-phenanthroline complex with the color of orange, but if H₂O₂ radicals exists in that reaction, Fe²⁺-tri-phenanthroline complex would not be formed. When the scavenger of H₂O₂ presence in the mixture, it will form the Fe²⁺-tri-phenanthroline complex (Utami *et al.*, 2017; Prahastuti *et al.*, 2020).

The results can be seen in Figure 1, Figure 2, Table I and Table II. The Median Inhibitory Concentration 50 (IC₅₀) was determined using linear regression analysis. Based on the result, PBEE exhibit high antioxidant activity for DPPH scavenging than BBEE which IC₅₀ PBEE

Table I. Phytochemical screening results of PBEE and BBEE

Compounds Group	Extract	
	PBEE	BBEE
Alkaloids	-	-
Flavonoids	+	+
Polyphenols	+	+
Saponins	-	-
Steroid/Triterpenoid	-/-	-/-
Terpenoids	+	+

(+) = contains the compounds group; (-) = does not contain the compounds group

Table II. The linear equation, coefficient of regression (R²) and IC₅₀ for DPPH scavenging activity of PBEE and BBEE

Samples	Linear Equation	R ²	IC ₅₀ (µg/mL)
PBEE	0.2404x + 22.278	0.9997	115.32
BBEE	0.1547x + 24.858	0.9936	162.52

Note: The assay was performed in triplicate. The equation was presented from linear regression analysis of the mean scavenging percentage

Table III. The linear equation, coefficient of regression (R²) and IC₅₀ for H₂O₂ scavenging activity of PBEE and BBEE

Samples	Linear Equation	R ²	IC ₅₀ (µg/mL)
PBEE	0.0791x + 10.677	0.9892	624.80
BBEE	0.0791x + 0.5782	0.9989	497.13

Note: The assay was performed in triplicate. The equation was presented from linear regression analysis of the mean scavenging percentage

115.32 µg/mL and BBEE 162.52 µg/mL classified moderate activity with IC₅₀ 101-250 µg/mL (Marjoni and Zulfisa, 2017). In line with DPPH scavenging activity, PBEE exhibit lower antioxidant activity for H₂O₂ radicals scavenging than BBEE which IC₅₀ PBEE 624.80 µg/mL classified inactive activity with IC₅₀ >500 µg/mL (Marjino and Zulfisa, 2017) and BBEE 497.13 µg/mL classified weak activity with IC₅₀ <500 µg/mL (Marjoni and Zulfisa, 2017). It means PBEE has more active antioxidant activity in DPPH scavenging activity than BBEE. BBEE has more active antioxidant activity than PBEE in H₂O₂ scavenging activity. It was in line with previous study that unripe plantain banana has IC₅₀ at 0.31 ppm while banana blossom has IC₅₀ at 21.02 ppm (Schmidt *et al.*, 2015; Liyanage *et al.*, 2015).

Anticancer potential testing was carried out in vitro on MCF-7 breast cancer cell lines using the MTS assay methods. MTS assay is the reduction of tetrazolium compounds by formazan compounds that are soluble in cell culture medium. This change was catalyzed by nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) produced by the

enzyme dehydrogenase in the living cell's mitochondrial respiration chain. The higher the cell viability, the more colored formazan formed which is characterized by the intensity of the color that is formed the more concentrated (Widowati *et al.*, 2013a; Widowati *et al.*, 2013b; Evacuasiyany *et al.*, 2014; Widowati *et al.*, 2015; Widowati *et al.*, 2016).

The results of MTS assay was shown in Figure 3. It shown that the sample was influenced the viability of MCF-7 cells. The higher concentration of the sample could decrease the viability cell which means inhibited the proliferation strongly. The IC₅₀ value of anticancer activity both PBEE and BBEE the data of inhibition activity was analyzed using probit analysis. The IC₅₀ values indicates sample concentration inhibit 50% of cell proliferation. The IC₅₀ value for PBEE and BBEE was found to be 115.00 µg/mL and 338.47 µg/mL respectively which means PBEE has more active anticancer classified moderate activity (IC₅₀ : 101-250 µg/mL) than BBEE classified weak activity (IC₅₀ : 250-500 µg/mL) (Marjoni and Zulfisa, 2017). Based on IC₅₀ values, PBEE has a moderate anticancer and BBEE has weak

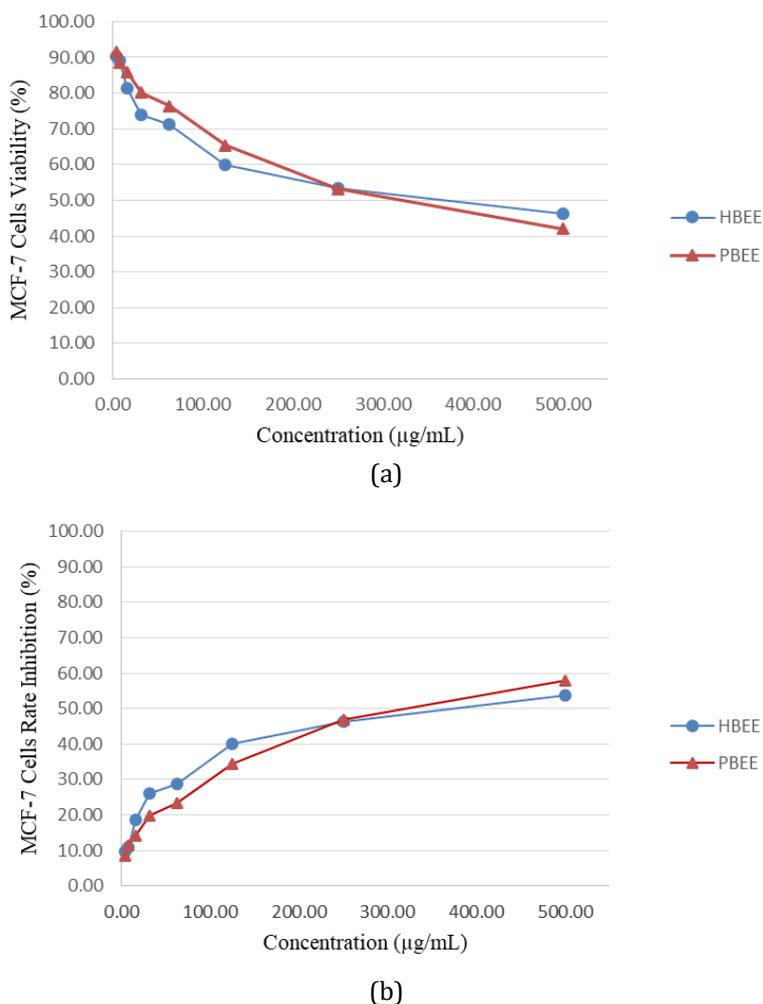


Figure 3. Cytotoxic activity for BBEE and PBEE in terms of (A) MCF-7 cells viability and (B) MCF-7 cells inhibition.

*The activity was presented as mean. The assay was done triplicate for each concentration and sample. Sample concentrations: 3.91 µg/mL; 7.81 µg/mL; 15.63 µg/mL; 31.25 µg/mL; 62.50 µg/mL; 125 µg/mL; 250 µg/mL; and 500 µg/mL.

anticancer toward MCF-7 breast cancer cells. This research showed that crude extract yielded anticancer in moderate and weak activities, its because the crude extract consists of several compounds had lower bioactivities compared to its compound, this result data was in line with previous study that fractionation, purification yielded fractions, compounds with higher antioxidant and bioactivity than crude extract (Barbosa-Pereira *et al.*, 2013).

Although the anticancer potential of PBEE and BBEE are moderate and weak activities, PBEE and HBEE contains secondary metabolites of flavonoid compounds. Flavonoids are compounds that known to have proliferative inhibitory activity

in various cancer cells and able to induce apoptosis. Catechins are belonging to flavonoid compounds that can reduce murine lymphoma cell proliferation mediated by apoptosis at concentrations above 100 µg/mL (Mooney *et al.*, 2002). Epicatechin suppresses human-induced dermal microvascular endothelial cell proliferation. In addition, epicatechin and related compounds have been found to modulate the signaling enzyme activity during angiogenic signaling, control its release and regulate its receptor (Lim, 2012). According to previous research, epicatechin has less significant effect on MCF-7 and T47D breast cancer cell proliferation compared to other catechins or flavan-3-ols such

as (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), and (-)-gallocatechin gallate (GCG) (Evacuasiy *et al.*, 2014).

Meanwhile, quercetin is also a flavonol group compound that can induce apoptosis of cancer cells by stimulating cytochrome c release from mitochondria to cytoplasm (Lim, 2012). Cytochrome c was known as heme protein that acts as a water-soluble electron carrier in mitochondrial oxidative phosphorylation. If an electron coil occurs through cytochrome c oxidase, ionic strength would change and causes the matrix waves. The cytochrome c exits into the cytoplasm because the nonspecific inner membrane permeability transition pore was open. It would binds to Apaf-1 and form a CARD (Caspase Recruitment Domain). Several CARDS was combined and formed the apoptosome complex that could bind pro-caspase-9 and activated it into caspase-9. Caspase-9 will later activates caspase-3 as the executor that will damage the nucleus and then degrade the DNA chromosomes of cancer cells (National Cancer Institute, 2001).

Quercetin also known could modulating various cell cycle regulators including p21, p27 (regulator protein inhibits the cell cycle) and Cyclin D1 (an important regulator of cell cycle development G1 to S phases) and stop the cancer cell cycle. Quercetin causes cessation of the cycle cells by increasing p21 expression and decreasing Cyclin D1 expression (Nunez *et al.*, 1998).

Research on the anticancer potential of banana peel (*Musa sapientum*) by Dahham (2015) states that PBEE has a cytotoxic effect on HCT-116 cells with a percentage inhibition of 85.32% at a 100 µg/mL concentration which indicates an antiangiogenesis effect by inhibiting microvascular growth of cancer cells. Research on anticancer potential of banana blossom (*Musa paradisiaca*) by Nadumane & Timsina (2014) states that BBEE has a significant cytotoxic effect on HeLa cells with IC₅₀ 20 µg/mL and extract fraction has maximum anti-proliferative effect with IC₅₀ value < 10 µg/mL. BBEE can induce apoptosis in HeLa cells (cervical cancer epithelial cells) through the activity of caspase-9 initiator. There was a two-fold increase in caspase activity in fraction HeLa cells compared with control HeLa cells, which confirmed the apoptosis induction of cell death in HeLa cervical cancer cell lines (Emami *et al.*, 2016).

CONCLUSION

Based on the result, PBEE and BBEE have moderate - high in DPPH scavenging and inactive - weak H₂O₂ scavenging activities. PBEE and BBEE have weak - moderate anticancer. PBEE has more active antioxidant activity in DPPH scavenging

activity than BBEE. BBEE more active antioxidant activity than PBEE in H₂O₂ scavenging activity. PBEE has more active anticancer than BBEE.

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