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Research Article
Anti-proliferation and Apoptosis Induction of Aqueous Leaf Extract of *Carica papaya* L. on Human Breast Cancer Cells MCF-7

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Abstract

Background and Objective: Breast cancer is the most frequently diagnosed cancer in women. Chemotherapy is the main method of breast cancer treatment but there are side effects. *Carica papaya* leaves is vegetable foods consumed by most people of Indonesia have potential as anticancer. The aim of this study was to investigate anti-proliferative and apoptotic induced effect of aqueous papaya leaves extracts on human breast cancer cell lines MCF-7. **Materials and Methods:** Inhibitory on cell proliferation was measured by MTT assay while apoptosis induction was measured using Annexin V. **Results:** The results showed that papaya leaf can inhibit the proliferation of human breast cancer cells MCF-7 with IC_{50} in 1319.25 μg mL^{-1}. The IC_{50} values of papaya leaf extract was higher than the IC_{50} value quercetin and doxorubicin. Papaya leaf extract can also induce apoptosis of breast cancer cells MCF-7 about 22.54% for concentration 659.63 μg mL^{-1} and about 20.73% for concentration 329.81 μg mL^{-1}. The percentage of cell apoptosis of papaya leaf extract lower than doxorubicin but higher than quercetin. **Conclusion:** This study indicated that papaya leaf extract have potential as anticancer through mechanism anti-proliferation and apoptosis induction.

Key words: *Carica papaya*, leaf, breast cancer, proliferation, apoptosis, extract, MTT assay, MCF-7 cell line

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cancer is a degenerative disease that is a public health problem in the world. Based on the results of RISKESDAS was known cancer incidence in Indonesia reached 1.4% per 1,000 respondents[1]. Breast cancer is the most frequently diagnosed cancer in women[2]. Chemotherapy is the main method of breast cancer treatment. However, since there are side effects of anticancer drugs[3], natural products such as herbs have been used as an alternative therapy[4]. Moreover, consumption of phytoestrogens found in foods such as soy beans is associated with a lower risk of breast cancer[5].

Carica papaya leaf has been used as a folk medicine for a varietyof ailments such as healing of burns, relief of asthma symptoms, treatment of intestinal worms, treatment of digestion problems, fever control and treatment of amoebic dysentery[6-8]. Papaya leaf has also been used to increase appetite, ease menstrual pain and relieve nausea[9]. A recent study found that papaya leaf extract could prevent growth of cancer cells including breast cancer[10]. However, because the study investigated all cancer cells not specific cancer cell and the study only tested cytotoxic activity, this study will investigate specific breast cancer cell lines and also tested the apoptoticinduced. The aim of this study was to test anti-proliferative and apoptotic induced effect of papaya leaves extracts on human breast cancer cell lines MCF-7.

MATERIALS AND METHODS

Material: *Carica papaya* leaves are used in this study were obtained from local farmers in Yogyakarta with variety Grendel. Sampling of *Carica papaya* leaves in this study based on observation of the number of leaves in the tree then divided three parts. Number of papaya leaves in every tree ranged between 18-25 leaves. Papaya leaves sample taken from the 7th or 8th.

Sample preparation: Papaya leaves are dried in an oven of 60°C for 3 h 3 times then milled. About 1 g of dried papaya leaves milled put in 20 mL of solvent and stirred. Solvents used in this study are methanol, ethanol 70% and water. The extraction method used is the percentage watt microwave/heat by 50% by setting 4 sec on and 60 sec off for three times then performed filtering using Whatman No. 1 filter paper and then stored in a refrigerator for further testing[11].

Determination of cell proliferation: Inhibitory of cell proliferation test was performed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazoliumbromide (MTT) assay according to Ebada *et al.*[12]. The MCF-7 cell (breast cancer cell) were cultured in DMEM medium, supplemented with 10% fetal bovine serum, 1% fungi zone and 2% penicillin-streptomycin. The cells were maintained at 37°C in a moisture saturated atmosphere containing 5% CO_2. The cells were seeded at density of 2×10^4 cells per well in 100 µL of medium and allowed to attach overnight. After the cells were grown to about 80% confluence, treatments were initiated by supplementing serial dilution of 3000 µg mL^{-1} for papaya leaves extract, 100 µg mL^{-1} for quercetin and 10 µg mL^{-1} for doxorubicin. All treatments were conducted in three replicates. The inhibition percentage of cells growth were calculated with formula: A-B/A×100%, where A = Control cell absorbance, B = Compounds absorbance, the inhibition concentration 50 (IC_{50}) value is defined as the concentrations of compound which inhibited 50% of the cell growth. The IC_{50} value was determined by regression linear equation.

Determination of apoptotic cell: Determination of apoptotic cell was conducted using Annexin Vstaining kit according to Elmore[13]. Briefly, the MCF-7 cell was seeded at a final density of 5×10^5 cells per well in 3 wells micro culture and incubated for 12 h in CO_2 incubator (37°C, 5% of CO_2 flow). The extract were added to cells at 659.63 and 329.81 µg mL^{-1} and incubated for 24 h. After 24 h of incubation, cells were harvested by the addition of tryps in, centrifuged for 5 min at 1000 rpm and finally washed with PBS. Finally, the cells were resuspended in 100 µL of Annexin-V-FLUOS staining kit (Roche) then incubated in dark room for 10 min at 20-25°C. Typical histogram of apoptotic cell was performed using flow cytometer. Doxorubicin was used as a positive control.

RESULTS

The inhibition of proliferation of MCF-7 cells were tested by MTT assay *in vitro*. The principle of this method is reacting the bioactive compound with cancer cells. Conversion of tetrazolium salt (MTT) into formazan blue is found only in cells that are still alive and the amount of formazan produced is proportional to the number of existing living cells. Thus MTT assay was used to test potential anti-proliferative activity of the extract[14]. The percentage of death of human breast cancer cells MCF-7 with serial dilution of papaya leaf extract 3000 µg mL^{-1}, quercetin 50 µg mL^{-1} and doxorubicin 6.25 µg mL^{-1} for 24 h incubation shown in Fig. 1-3.

This study shows the percentage of MCF-7 cell death is influenced by the concentration of the extract or samples. The greater concentration given on MCF-7 cells the number of

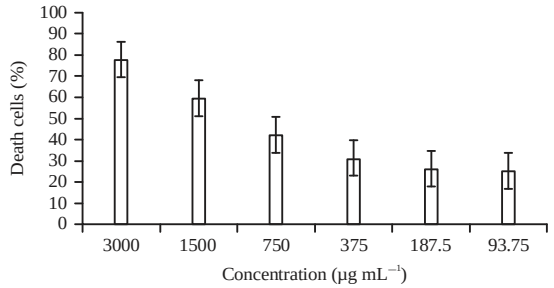

Fig. 1: Percentage death cells of MCF-7 by aqueous papaya leaf extract

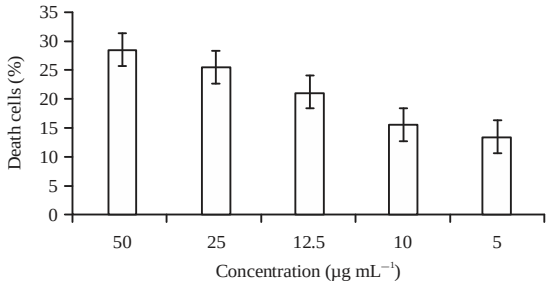

Fig. 2: Percentage death cells of MCF-7 by quercetin

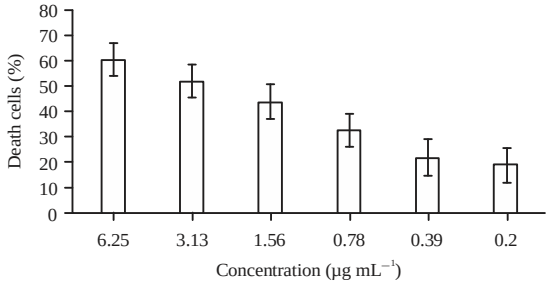

Fig. 3: Percentage death cells of MCF-7 by doxorubicin

Table 1: Inhibitory concentration 50% (IC_{50}) value of extract, quercetin and doxorubicin

Samples	IC_{50} (µg mL^{-1})
Extract	1319.25
Quercetin	111.36
Doxorubicin	3.87

dead cells increased. The results showed that the aqueous extract of papaya leaves has potential as an anti-proliferation in MCF-7 cells as evidenced by the increasing number of cell death compared to control.

Inhibition of aqueous papaya leaf extract on proliferation breast cancer cells MCF-7 was indicated by inhibitory concentration (IC_{50}) value (Table 1). The IC_{50} value were calculatedby the linear regression equation from the data of Fig. 1-3. The linear regression equation obtained is:

Table 2: Percentage of apoptotic of human breast cancer cells MCF-7 by extract and doxorubicin

Samples	Dose (µg mL^{-1})	Apoptotic (%)
Extract	659.63	22.54±0.21
	329.81	20.73±0.06
Quercetin	55.68	3.48±0.18
	27.84	3.26±0.13
Doxo	1.94	86.80±0.22
	0.97	78.07±0.25

Data were expressed as Mean±SD, (n = 3)

$$y = 0.0186x+25.462 \text{ with } r = 0.9676$$

The IC_{50} value of aqueous extract of papaya leaf calculated using the equation was 1319.25 µg mL^{-1}. The IC_{50} of quercetin and doxorubicin were also measured for comparison as positive control. Quercetin is a flavonoid compounds contained in papaya shoots[15] while doxorubicin is a chemotherapeutic drug commonly given for breast cancer treatment. The IC_{50} value of quercetin found 111.36 µg mL^{-1} and IC_{50} value of doxorubicin found 3.87 µg mL^{-1}. The IC_{50} values of papaya leaves extract is higher than quercetin and doxorubicin. It means that papaya leaf extract has anti-proliferative potency lower than quercetin and doxorubicin.

The induction of apoptosis in MCF-7 cells by the aqueous extract of *Carica papaya* L., leaves was monitored using flow cytometry. Results demonstrated the occurrence of cell death in MCF-7 cells following addition of the extract to the culture medium. Apoptosis was induced when cells were treated with 659.63 and 329.81 µg mL^{-1} extract. The percentage of apoptotic of human breast cancer cells MCF-7 shown in Table 2.

Carica papaya leaves extract can induce apoptosis of MCF-7 cell line 22.54% for concentration 659.63 µg mL^{-1} and 20.54% for concentration 329.81 µg mL^{-1}. The percentage of apoptotic of MCF-7 by papaya leaves extract is lower than doxorubicin but is higher than quercetin.

DISCUSSION

Potential of papaya leaves extract as antiproliferation of breast cancer cells is suspected due to the content of bioactive compounds flavonoids. Based on preliminary research it is known that papaya leaf extract containing flavonoids quercetin of 3.39 mg g^{-1} and papaya leaf extract also has antioxidant activity. Antioxidants are known to reduce the risk of cancer in both research laboratory and epidemiological research through its ability to reduce damage caused by free radicals[16]. Supplements of vitamin C and E in breast cancer

shown to cause cell differentiation and apoptosis and inhibit tumor progression[17-20]. The results of epidemiological studies have shown that antioxidant supplements may reduce the risk of breast cancer recurrence or prevent the growth of breast cancer[21]. Rapid changes in diet and lifestyle, may influence heritability of the variant phenotypes that are dependent on the nutraceutical or functional food supplementation for their expression. It is possible to recognize the interaction of specific nutraceuticals, with the genetic code possessed by all nucleated cells[22].

Mechanism of papaya leaf extract to inhibit the proliferation of breast cancer cells MCF-7 allegedly through the reduction of Reactive Oxygen Species (ROS) by antioxidant compounds contained in papaya leaf extract impact on the decrease of nuclear factor-κB (NF-κB) and impact on the gene expression associated with oxidative stress in MCF-7 cells such as COX-2, AP-1, Bcl-2 and Bcl-XL which can inhibit the proliferation of breast cancer cells MCF-7[23-27]. Decrease of NF-kB also resulted in decreased the expression of cyclin D1 and increased tumor suppressors such as p27, p21 and p53, causing cell cycle arrest which inhibit cancer cell proliferation[28-33]. The IC_{50} value can be predicted by ligand-receptor binding interactions for protein kinase (CK2) employing quantitative structure active relationship (QSAR) model[34].

Cells die by apoptosis when cells become old or damaged, necrosis or a combination of the two and are replaced with new cells. On the other hand, cancer cells are immortal since they are resistant to apoptosis. Chemotherapy kills cancer cells through apoptosis and/or necrosis[35]. Potential of aqueous extract of papaya leaves in triggering apoptosis probably caused by flavonoids. Flavonoids can stimulate apoptosis through multiple mechanisms include inhibition of the activity of DNA topoisomerase I/II, modulation of signaling pathways, decreasing gene expression of Bcl-2 and Bcl-XL, increasing the gene expression of Bax and Bak as well as the activation of endonucleases[36]. Apoptosisor type 1 programmed cell death is a normal physiological process, however defects in apoptosis is a major cause of cancer[13]. The apoptotic mechanism is often used as a criterion for discovering new anticancer agents. Several natural compounds such as quercetin and curcumin have shown apoptotic-inducing properties[37]. Apoptosis is characterized by specific morphological and biochemical changes of cells, including cell shrinkage, nuclear condensation and DNA fragmentation, dynamic membrane blebbing and loss of cell adhesion, phosphatidylserine externalization and intracellular specific proteolysis[38-41].

CONCLUSION

In conclusion, our data indicated that the *Carica papaya* leaves extract specifically reduced viability of human breast cancer cells MCF-7. *Carica papaya* leaves extract have potential as anti-proliferative and apoptotic induction. However, the mechanism of the action is still unclear. Thus, further investigations including isolation of individual active flavonoid and elucidation of the molecular mechanisms involved are needed to fully understand the active ingredient and potential of *Carica papaya* leaves as a chemopreventive food.

SIGNIFICANT STATEMENTS

Cancer is a degenerative disease that is a public health problem in the world. Breast cancer is the most frequently diagnosed cancer in women. Chemotherapy is the main method of breast cancer treatment but there are side effects. *Carica papaya* leaf is vegetable that usually consumed by Indonesian people. A recent study found that papaya leaf extract could prevent growth of cancer cells including breast cancer. Because the study investigated all cancer cells and only tested cytotoxic activity. This study will investigate specific breast cancer cell lines and also tested the apoptotic induced. This study can solve the problem for women to prevent breast cancer and for breast cancer patient to inhibit cancer cell proliferation by functional food.

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