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Research Article

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Cytotoxicity activity of male *Carica papaya* L. flowers on MCF-7 breast cancer cells

Marline Nainggolan[1*] and Kasmirul[2]

[1]*Department of Pharmaceutical Biology. Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia*
[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia*

Abstract

New approach of breast cancer therapy is developed toward using natural product as chemotherapy. Male Carica papaya L. flowers is one of natural product that have many pharmacology activity include anticancer. This research to evaluate the effects of ethanol extract and fractions (n-hexane, ethylacetate and water) of male Carica papaya L. flowers on cytotoxicity on MCF-7 cell lines. Cytotoxicity activity were evaluated using the MTT assay and have IC_{50} 55.875 µg/mL; 101.282 µg/mL; 148.692 µg/mL; 356.489µg/mL respectively.

Keywords: *Carica papaya* L, flowers, male, extract, fractions, MCF-7, MTT.

INTRODUCTION

The diversity of medicinal plants in Indonesia is one of chances in development potential of Indonesia in the globalization era [1]. The use of medicinal plants in the community is increasing in several decades [2,3]. Indonesia has thousands of islands with various plants in it and the manners of community using plants as treatment for every disease traditionally [1]. male *Carica papaya* L. flowers always consumed as vegetables. Breast cancer is a type of cancer that most often affects women and leading cause of death in women, and based on the US data in 2010 breast cancer is the most common cancer with 209.060 new cases [4].

In previous study, male *Carica papaya* L. flowers have been researched as antimutagenic from ethanol and ethylacetate extract [5,6], antioxidant [7], the study was attempted to evaluate cytotoxicity activity from extract and fractions of male *Carica papaya* L. flowers and obtain the characteristics of simplex .

EXPERIMENTAL SECTION

Plant material

Fresh flowers of *Carica papaya* L. were collected from Gunung Berkat village, Asahan regency, Sumatera Utara province, Indonesia. *Carica papaya* L. was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in herbarium. DMSO (Sigma), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma.

Characterization and determining class of chemical compound

Characterization of simplex include determination of water content, determination of water-soluble extract, determination of the ethanol-soluble extract, total ash content determination and the determination of ash-not dissolve in acid content. Determining the class of chemical compounds carried out on simplex, ethanol extract, *n*-hexane fraction, ethylacetate fraction, and water fraction [8,9]

Extraction and Fractionation

Briefly, 250 g of dried ground powder from *Carica papaya* L. was extracted using ethanol 96% with maceration method, filtrate was collected and then evaporated under reduced pressure to gave of viscous ethanolic extract and dried using freeze drying [10]. The extract was added with 100 mL aquadest to yield liquid form of ethanolic extract. The extract was fractioned with *n*-hexane, and ethylacetate.

Cytotoxicity assay

The ethanol extract and fractions were submitted to cytotoxicity test. In that way, MCF-7 cell line was grown in RPMI 1640 medium, medium containing 10% FetaL Bovine Serum (Gibco), 1% penicillin-streptomycine (Gibco), and fungizone 0.5% (Gibco) in a flask in a humidified atmosphere (5% CO_2) at 37^{o}C. The inoculums seeded at 10^4 cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated by ethanol extract and fractions. After incubation 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h in 37^{o}C. Viable cells react with MTT to produce purple formazan crystals. After 4 h, SDS 10% as stopper (Sigma) in 0.01N HCl (Merck) was added to dissolve the formazan crystals. The cells were incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaken, and absorbance was measured using ELISA reader at λ 595 nm. The data which were absorbed from each well were converted to percentage of viable cells [11, 12].

$$Percentage\ of\ viable\ cells = \frac{B - C}{A - C}\ x\ 100\%$$

Where A, B and C are absorbance of control group, treatment group and medium (vehicle), respectively.

Statistical analysis

All data were analyzed using regression using SPSS 20.

RESULTS AND DISCUSSION

Identification of papaya flowers was done in Research Centre for Biology, Indonesian Institute of Science, Bogor, is *Carica papaya* L. from caricaceae family.

Characterization and determining class of chemical compound results

Table 1. Characterization results of male *Carica papaya* L. flowers

No	Parameter	Simplex (%)	Ethanol extract (%)
1	Water content	7.32	15.33
2	Water-soluble extract content	19,25	59,50
3	Ethanol-soluble extract content	10,61	23,21
4	Total ash content	2,25	0,79
5	Ash-not soluble in acid content	0,22	0,10

The result determining the class of chemical compounds of papaya flowers simplex were shown presence of triterpenoids/steroids, flavonoids, tannins, and glycosides group compound. In *n*-hexane fraction was shown presence of triterpenoids/ steroids group compound, in ethylacetate fraction was shown presence of flavonoids, tannins and glycosides group compound, and in water fraction was shown presence of glycosides group compound.

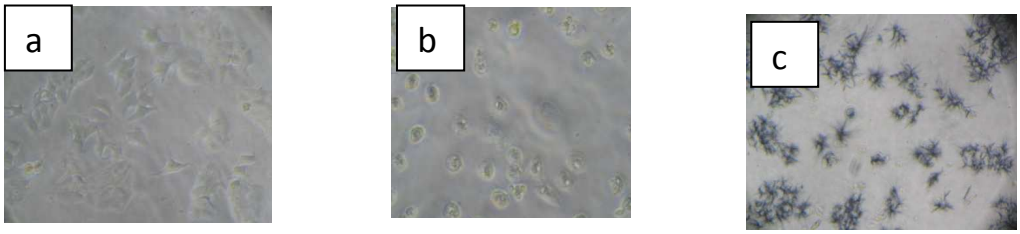

Figure 1. Effect of ethanol extract on MCF-7 cells and formazan crystal form

a. Control (MCF-7 cells without treatment)
b. ethanol extract 125 µg/mL
c. formazan crystal on MCF-7 cells treatment with ethanol extract 125 µg/mL

The study was aimed to evaluate the activity of ethanolic extract and fractions as chemotheraphy. In the study, ethanolic extract and fractions were evaluated for their cytotoxic effects on MCF-7 breast cancer cell lines. Cell viability was determined by MTT assay after an incubation for 24 h. Morphological change of the cells were more extensive after treatment with ethanolic extract and fraction.

Table 2. Cytotoxcicity results of ethanol extract and fractions from papaya flowers (*Carica papaya* L.)

No	Name	IC_{50} (µg/mL)
1	Ethanol extract	55.875
2	*n*-hexane fraction	101.282
3	Ethylacetate fraction	148.692
4	Water fraction	356.489

Extract that having IC_{50} values <100 µg/mL categorized as potent extract [13,14]. Previously has been done determining class of chemical compound from extract and fraction and finded chemical compound like steroids/triterpenoids, flavonoids and saponins that having anticancer activity.

From the test results and calculation of IC_{50} values extract and fractions, then that can be categorized into potent extract and fractions, there is ethanol extract because their IC_{50} values below 100 µg /ml.

Triterpenoid and steroid compounds have activity to healing inflammation, proliferation, apoptosis, invasion, metastasis, and angiogenesis. Since many of these compounds showed good potential in dealing with a variety of cancers mechanisms, such as regulation of transcription factors regulate (eg, nuclear factor-kappaB [NF-κB], anti-apoptotic proteins (eg, bcl-2, bcl-xL), the originator of cell proliferation metalloproteinases [MMPs], intracellular adhesion molecule-1 (ICAM-1), and angiogenic proteins (vascular endothelial growth factor (VEGF) [15].

Flavonoid compounds inhibit cell proliferation in various human cancer cells through the inhibition of oxidative processes that can lead to cancer initiation. This mechanism is mediated decrease xanthin oxidase enzyme, Cyclooxygenase (COX) and Lipooxygenase (LOX) required in the process prooxidation thereby delaying cell cycle. Flavonoids also inhibit the expression of topoisomerase I and II enzymes that play a role in catalyzing DNA screening. Topoisomerase enzyme inhibitor complex will stabilize DNA topoisomerase and cause cuts and damage [16].

Saponins can recognize cancer cells, because cancer cells have cell membranes and structures are different from normal cells. Cancer cell membranes contain more compounds such as cholesterol. Saponins can bind cholesterol contained in the membrane of cancer cells, thereby disrupting membrane permeability [17]. Saponins also reduce the occurrence of reactive oxygen species such as H_2O_2 and inhibit signaling pathways phosphatidyl inositol-3 kinase which may be the reason for the prevention of damage chromosome [18].

The male *Carica papaya* L. flowers are used in North Sumatera to treat fever, and as vegetables. Although some compound have been identified as possesing medicinal properties, none of these compounds has ever reached clinical trials. Moreover, the anticancer effect of male *Carica papaya* L. flowers have not been validated in vitro to date based on their use in Indonesia or other system of medicine.

The cytotoxicity estimate of natural product is related to content of active compound in these plants including *Carica papaya* L. Flavonoids, saponins and triterpenoids/steroids estimated as active compound. We were evaluated the activity of ethanol extract and n-hexane, ethylacetate and water fractions on cytotoxicity.

However, the selectivity index and molecular mechanism of apoptosis induction and cell cycle modulation by this extract and fractions need to be explored more detail. Based on the results, we were concluded that ethanol extract of male *Carica papaya* L. flowers is potential to developed as co-chemotherapeutic agent in breast cancer therapy.

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