

Cytotoxic Potential of Flavonoid from *Nicotiana tabacum* Leaves on MCF-7 Human Breast Cancer Cells

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Abstract

Flavonoid has potential bioactivity as anticancer agents. The flavonoid of cultivated tobacco (*Nicotiana tabacum*), locally known as “Kasturi”, leaves was screened for its cytotoxicity against MCF-7 human breast cancer cells and non-transformed Vero cells (African normal cell kidney line) in different concentrations. This study aimed to examine the cytotoxic potential of the flavonoid of Kasturi tobacco leaves against MCF-7 human breast cancer cells. Flavonoid obtained from methanolic extracts of Kasturi tobacco leaves, which have been purified from nicotine. The flavonoid of Kasturi tobacco leaves with concentrations of 20 to 640 µg/mL were exposed to MCF-7 and Vero cells for 24 h. Cell viability was evaluated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Flavonoid of Kasturi tobacco leaves with concentrations of 160 µg/mL decreased the MCF-7 cell viability more than 50%, with an inhibitory concentration 50 (IC₅₀) value of 148.41 µg/mL. Meanwhile, it inhibited 50% of Vero cell viability at 255.35 µg/mL. The flavonoid of Kasturi tobacco leaves has cytotoxic activity on MCF-7 cells, and might be a potential alternative agent for human breast cancer therapy.

Keywords: *flavonoid, tobacco leaves, human breast cancer cells, anticancer activity.*

INTRODUCTION

Cultivated tobacco, locally known as Kasturi tobacco, (*Nicotiana tabacum*) is local tobacco *Voor Oogst*, which is planted in Jember and Bondowoso areas. Kasturi tobacco is intended as raw material for domestic cigarettes, which reaches 88.64% and around 11.36% is exported. In addition to extensive use of cigarette products, tobacco leaves are a rich source of many biologically active substances with pharmacological properties. Many researchers have made efforts to diversify tobacco products, starting with determination of

total phenolic and total flavonoid from tobacco leaves (Fathiazad, *et al.*, 2006; Xie, *et al.*, 2011; Karabegović, *et al.*, 2011), and analysis the antioxidant activity of purified flavonoid from nicotine and phenolic acid (Docheva, *et al.*, 2014). On the other hand, it is known that flavonoid have various anticancer phytochemicals with potential bioactivity that can reduce cancer susceptibility,

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and can be candidates for cancer prevention (Yang, *et al.*, 2001; Le Marchand, 2002).

Breast cancer is the second most common malignant tumor in the world and causes cancer-related deaths after lung cancer. Unfortunately, the overall response rate of cancer treatment is unsatisfactory because of the late diagnosis and efficacy of poor treatment, especially resistance to chemotherapy drugs and metastasis to other organs (Metwaly, *et al.*, 2012). In addition, many chemotherapy drugs reduce the therapeutic effect due to drug resistance problems (Peters, *et al.*, 2002). Chemotherapy drugs also cause toxicity to normal cell, and unpleasant side effects for patients. For this reason, it takes research and development of a new class of anticancer agents that have selective and efficient toxicity in cells. Among various sources of anticancer drugs, the use of natural resources from plants has many benefits about the potential to produce a variety of anticancer agents. In this study, we aimed to find a new source of natural anticancer isolated from Kasturi tobacco leaves and investigate the cytotoxicity ability of the flavonoid of Kasturi tobacco leaves against MCF-7 and Vero cells.

MATERIALS AND METHODS

Kasturi tobacco Leaves Extraction

Dried Kasturi tobacco leaves (50 g) were ground and sieved until tobacco leaf powder is obtained. Tobacco leaves powder were macerated 3 times with methanol (Merck, Darmstadt, Germany) and stirred for 24 h to bind compounds in tobacco leaves (Hossain & Salehuddin, 2013), then were filtered using a Buchner funnel. The filtrate was fractionated by liquid-liquid extraction using ethyl acetate (Merck) (Mujwah, *et al.*, 2010), and separated between the methanol and ethyl acetate phases with a separating funnel. The ethyl acetate phase was added with 10% HCl (Merck) to remove the nicotine (alkaloid) content and was tested with Dragendorff reagent (ACS Chemicals, Gujarat, India). The extract will be yellow if

it is free of nicotine and will be orange if it still contains nicotine. Extract was evaporated using a rotary evaporator until a thick extract is obtained. The extract was weighed 1.216 g, with a yield of 2.432%.

Total Phenolic in Kasturi tobacco Leaves Extract

The extract was dissolved with methanol (Merck) in a concentration of 1 mg/mL. The reaction mixture was made with a composition of 0.5 mL of extracted methanol solution, 2.5 mL of 10% Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, Missouri, USA) that has been dissolved in water and 2.5 mL of 7.5% NaHCO₃ (Merck). The blank was made with a composition of 0.5 mL of methanol (Merck), 2.5 mL of 10% Folin-Ciocalteu reagent (Sigma-Aldrich) dissolved in water and 2.5 mL of 7.5% NaHCO₃ (Merck). The sample was incubated at 45°C for 45 minutes. Absorbance was determined using a spectrophotometer at λ_{max} =765 nm and carried out in triplicate for each analysis. The same procedure was repeated for standard gallic acid solution (Sigma-Aldrich) as a calibration curve (Stanković, 2011). Based on the absorbance measured, the phenolic concentration is read (mg/mL) from the calibration line; then the phenolic content in the extract is expressed in the form of gallic acid equivalent (mg GA/g extract). The phenolic content in Kasturi tobacco leaves extract was 0.878 mg/g.

Cell Line and Culture Condition

Human breast cancer cells (MCF-7) and normal African green monkey kidney epithelial (Vero) cell lines were maintained at the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia. The cell culture medium was Dulbecco's modified Eagle's medium (DMEM) (Gibco, Canada, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Brazil, USA), 100 units/mL penicillin and 100 µg/mL streptomycin (Gibco, Germany). The cells were cultured at 37°C under a humidified atmosphere containing 5% CO₂.

Cytotoxicity Screening by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) Assay

Cytotoxicity of flavonoid was determined by MTT assay. Briefly, MCF-7 and Vero cells were seeded into 96-well plates (5000 cells/well in 100 μ L of media) and incubated for 24 h. Prior to the experiment, the stock solution of flavonoid was prepared by dissolving in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) and diluted to working solutions with media serially to obtain appropriate concentrations. The final concentration of DMSO (Sigma-Aldrich) in each sample did not exceed 1% v/v. Cells were treated with the flavonoid at concentrations of 20, 40, 80, 160, 320, and 640 μ g/mL and incubated for 24 h. MCF-7 and Vero cells in positive control group were treated with doxorubicin (Sigma-Aldrich). The medium from each well was removed carefully after incubation. Two hundred microliters of MTT solution (5 mg/mL) (Sigma-Aldrich) was added. The plates were incubated for 4 h in dark. After incubation, MTT solution was removed without disturbing the formazan crystals and 100 μ L of DMSO (Sigma-Aldrich) was added to each well and mixed well to dissolve the crystals properly. The development of purple color due to formation of formazan crystals visualized the viable cells. The absorbance was measured on microplate reader at 570 nm. The inhibitory concentration 50 (IC_{50}) values were determined from the graphs of flavonoid on MCF-7 and Vero cells. The cell viability percentage was calculated based on the absorbance ratio between cell culture treated with flavonoid and the untreated control multiplied by 100% (Wang, *et al.*, 2006). Experiment was repeated three times independently, and each with triplicate sets of reaction.

Statistical analysis

The mean values \pm standard deviation of three independent experiments was used for quantitative variables. Statistical significance was ascertained by one-way ANOVA, followed by Tukey's post hoc test of significance between

different groups. Statistical significance was predefined as $P \leq 0.05$.

RESULTS

The flavonoid of Kasturi tobacco leave was tested to evaluate the cytotoxic potential against the human breast cancer cells. The MTT assay showed remarkable cytotoxicity against MCF-7 cells in a dose dependent manner. The flavonoid started to show cytotoxicity on Vero cells at concentrations of 320 μ g/mL and the calculated IC_{50} was obtained at 255.35 μ g/mL. The cell viability count to determine anticancer activity against MCF-7 and Vero cells is represented in Table 1 and Figure 1. The anticancer activity of flavonoid against MCF-7 cell lines showed higher cytotoxicity at concentrations of 160, 320, and 640 μ g/mL, with an IC_{50} of 148.41 μ g/mL. These data suggested that the extracts are more toxic to cancer cells than normal cells. Nevertheless, flavonoid was not as active as doxorubicin. Doxorubicin with concentration of 0.8 μ g/mL can decrease cell viability of MCF-7 and Vero cells about 65.28 \pm 7.20 % and 58.92 \pm 6.90 %, respectively.

DISCUSSION

Cancer chemoprevention by using natural ingredients in the form of dietary or synthetic substances can reverse, suppress, or prevent carcinogenic development. This strategy is very interesting to combat the dogma associated with increasing cases of cancer throughout the world (Tsao, *et al.*, 2004). Plant extracts that act as anti-cancer drugs must be able to kill cancer cells without causing excessive damage to normal cells (Lacroix, *et al.*, 2006). Flavonoids are plant pigments consisting of natural phytochemical classes, and have various biological effects that can play a role in cancer prevention and cancer therapy. Increased anticancer activity correlates with an increase in the number of polyphenolic compounds (Danciu, *et al.*, 2015). Quercetin, a member of flavonol, is reported

Table 1. Effect of flavonoid of Kasturi tobacco leaves on cell viability of MCF-7 and Vero cells by MTT assay.

Concentration (µg/mL)	Cell Viability (%) ^a		P Value
	Vero Cell Line	MCF-7 Cell Line	
Flavonoid 640	6.98±2.03	1.54±0.77	0.56
Flavonoid 320	38.26±19.04	2.32±1.54	0.00
Flavonoid 160	93.52±17.54	39.77±27.27	0.00
Flavonoid 80	98.92±14.77	96.05±16.30	0.76
Flavonoid 40	105.33±8.67	101.51±14.90	0.68
Flavonoid 20	112.18±19.59	93.69±7.91	0.05
Doxorubicin 0.8	58.92±6.90	65.28±7.20	0.50

^aMean±standard deviation of three independent experiments; ^bnormal cells; ^ccancer cells. Data were analyzed using one-way ANOVA and followed by Tukey's post hoc test ($P \leq 0.05$).

as an attractive anticancer agent against breast cancer (Brusselmans, *et al.*, 2005; Du, *et al.*, 2010; Hashemzaei, *et al.*, 2017). The antioxidant activity of quercetin is believed to have a cytoprotective role against oxidative stress. Quercetin is not only protecting cells from free radical damage through antioxidant effects, but also motivates apoptotic cell death through pro-oxidant activity and inhibits tumorigenesis (Gibellini, *et al.*, 2010).

MCF-7 cells are estrogen-receptor (ER) positive and are classified as low-grade and luminal types (Kenny, *et al.*, 2007). Our results showed that the flavonoid of Kasturi tobacco leaves has more cytotoxic effects on MCF-7 than Vero cells (Table 1 and Figure 1). These results indicated that our extract is more selective for breast cancer cells than normal cells. Flavonoid of Kasturi tobacco leaves

can be considered as potential chemotherapy or chemopreventive agent based on its ability to induce apoptosis in cancer cells with relatively low toxicity to normal cells. The IC_{50} value of the flavonoid of Kasturi tobacco leaves (148.41 µg/mL) was found to be more efficient for anti-breast cancer cell activity. Therefore, anticancer strength may be related to quercetin content in our extract, which quercetin can induce cytotoxicity in the cancer cell line through various routes of action. Quercetin can induce DNA damage, which needs to be repaired before cell division occurs, and induces cytotoxicity by activating the intrinsic pathway of apoptosis (Srivastava, *et al.*, 2016). This confirms our assumption that the flavonoid can be responsible for the anticancer activity of Kasturi tobacco leaves.

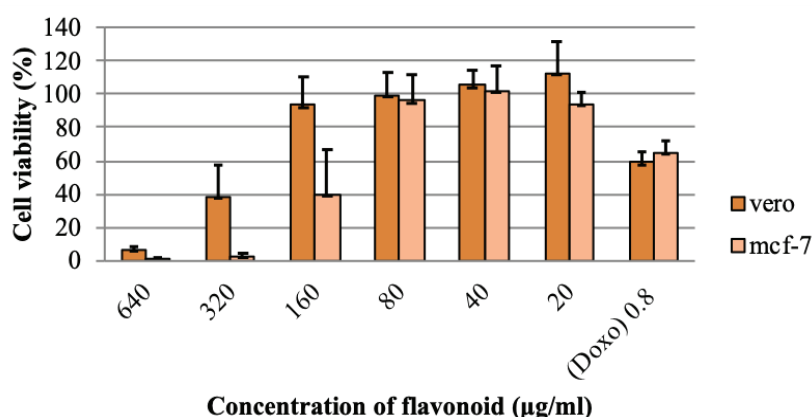


Figure 1. Cytotoxic potential of flavonoid from Kasturi tobacco leaves on MCF-7 and Vero cells. Error bar represents mean±standard deviation of experiments performed in three repetitions.

CONCLUSION

Viability of breast cancer cells will decrease according to dependent-dose. The flavonoid of Kas-turi tobacco leaves proved to be able to reduce 50% viability of breast cancer cells at a concentration of 148.41 µg/mL. The MTT assay showed remarkable cytotoxicity against MCF-7 cell. This flavonoid could be developed as an alternative chemopreventive agent, however further research should be pursued.

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AUTHORS' STATEMENT

The authors declare that they have no potential conflict of interest regarding submission and publication of this manuscript.

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