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RESEARCH ARTICLE

Cytotoxic activity of *Cantigi* leaf extract (*Vaccinium varingiaefolium Blume Miq.*) on HeLa cervical cancer cells and A549 lung cancer cells

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Keywords
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Abstract

Background: Cervical and lung cancer cases in 2018-2020 have increased worldwide and in Indonesia. Cancer treatment often involves multidisciplinary approaches and is uncomfortable. Therefore, alternative medicine from natural ingredients is needed. Previous studies showed that the Cantigi leaf ethyl acetate extract has cytotoxic activity categorised as very strong on L1210 leukaemia cells (IC_{50} of 8.29 ppm) and strong on MCF-7 cells (IC_{50} of 75.23 ppm) and T47D cells (IC_{50} of 88.89 ppm), but no reports exist on HeLa and A549 cells. **Objective:** To determine the cytotoxic activity of the ethyl acetate extract of Cantigi leaf on HeLa cells and A549 cells. **Methods:** Powdered dry young Cantigi leaves were macerated with hexane, then ethyl acetate. After vacuum evaporation, the dry extract was characterised and tested for its cytotoxic activity on HeLa cells and A549 cells using the MTT method. **Results:** The ethyl acetate extract colour was dark green with a distinctive Cantigi odour; it had a water content of 4.3% and an acidic pH of 2.87. It showed functional groups of O-H, C-H (aliphatic), C-H (hydrocarbon), C=N, C=O, and C=C. Finally, the IC_{50} of the ethyl acetate extract on A549 cells was 74.74±5.29 ppm, while on the HeLa cells, it was 79.69±2.54 ppm. **Conclusion:** The ethyl acetate extract of Cantigi leaves showed potent in vitro cytotoxic activity on HeLa cervical cancer cells and A549 lung cancer cells.

Introduction

In 2020, cancer became the first cause of death worldwide (about 10 million deaths), with new cases of lung cancer and cervical cancer of 2.21 million and 604,000, respectively, followed by death cases of lung cancer (1.80 million deaths) and cervix cancer (342,000 deaths) (Pelosci, 2021; WHO, 2021). In Indonesia, cervical cancer increased by about 15% compared to 2018 (approximately 36,633 cases) and caused 12,000 deaths (IARC, 2020). The type of treatment needed depends on the type and stage of cancer and may require more than one cycle of treatment (ACS, 2021a). In general, the side effects of cancer therapies cause painful and uncomfortable conditions; therefore, complementary and alternative medicine (CAM), including herbal medicine, have become common medical products and practices (NCI, 2021).

Also known as Cantigi, the *Vaccinium varingiaefolium* Blume Miq. is an endemic plant in West Java province, Indonesia. It grows well, especially near the crater of volcanoes, spreading from the west to the east part of Indonesia. The authors' first research showed that the ethyl acetate extract of Cantigi leaves contained flavonoids, steroids, tannins, and triterpenoids. The cytotoxic activity of the leaf extracts in vitro on cancer cells is categorised as very strong on leukaemia L1210 cells (Kosasih, Yulyana & Winarno, 2016) and strong on breast cancer T47D and MCF-7 cells, but relatively weak on normal Vero cells (Kosasih *et al.,* 2019). Based on cancer problems and previous reports, it was deemed essential to conduct this study to determine the cytotoxic activity of the ethyl acetate extract of Cantigi leaves on HeLa cells and A549 cells.

Material and methods

Materials, chemicals, and reagents

Young red Cantigi leaves were taken from Mount Tangkuban Parahu, Bandung, West Java, Indonesia, and determined at the Indonesian Institute of Sciences (LIPI), Cibinong, West Java, Indonesia. All chemicals and reagents used were analytical grades.

Extract preparation

Young red leaves (5 kgs) were washed, dried at 40°C, powdered, and macerated with hexane at room temperature for 24 hours. After filtration, the residual product was dried then macerated with ethyl acetate for another 24 hours. The filtrate was dried using a vacuum evaporator, and then the dried extract was evaluated for specific and nonspecific parameters (Ditjen POM, 2000), the Fourier transform infrared (FTIR) analysis (Nithyadevi and Sivakumar, 2015), and the MTT assay (Kosasih *et al.,* 2019).

MTT assay

HeLa cells and A549 cells were purchased from Laptiab BPPT, the Agency for the Assessment and Application of Technology, Serpong, Banten, Indonesia. The cells were grown in a media supplement with 10% foetal bovine serum harvested using trypsin, then counted using a haemocytometer. A density of 5000 cells per well was suspended in 100 µL medium in a microwells plate and placed in an incubator. After one day, the plated cells with 100 µL medium of a series of extract concentrations in Dimethylsulfoxide (DMSO), all done in triplicate. After 24 hours, the culture was washed twice with phosphate buffer saline, and the medium was changed. After that, 100 µL MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] was poured and maintained in an incubator for four hours to convert MTT to formazan crystals by reacting it with metabolically active cells. After the addition of sodium dodecyl sulfate, the viable cells were measured at 570 nm using a plate reader. The IC_{50} of samples was determined using a linear regression curve derived from concentration log versus % cell viability. Doxorubicin was used as a positive control (Kosasih *et al.,* 2019). The measurements were all in triplicate.

Results

Specific and nonspecific parameters data of *Cantigi* leaf can be seen in Table I and Table II.

Table I: Specific parameters data of the ethyl acetate extract of *Cantigi* leaf

Parameter	Description
Organoleptic:	
Colour	Dark green
Odour	*Cantigi* specific
Taste	Slightly bitter
Form	Powder

Table II: Nonspecific parameters data of the ethyl acetate extract of *Cantigi* leaf

Parameter	Description
Water-soluble content	5.61±0.14 %
Total ash content	0.37±0.01 %
Total flavonoid content	1.034±0.021%
Total phenol content	12.52±0.31 %
pH	2.87±0.03
Microbiology:	
Total plate count	Negative
Coliform	Negative
Yeast/mould	Negative
FTIR:	
Functional groups	Wavenumber (cm^{-1})
O-H	3415.78
C-H aliphatic	2938.00
C=O	1687.21
C-H	1450.46
C=C	697.41; 753.04
N-H	697.42

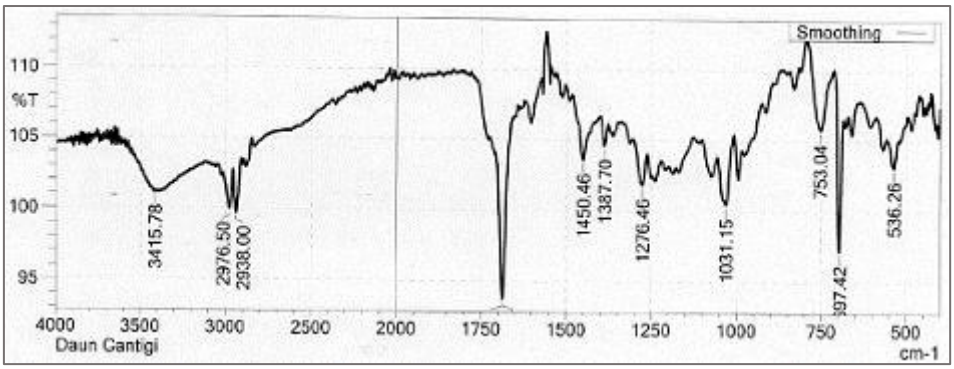
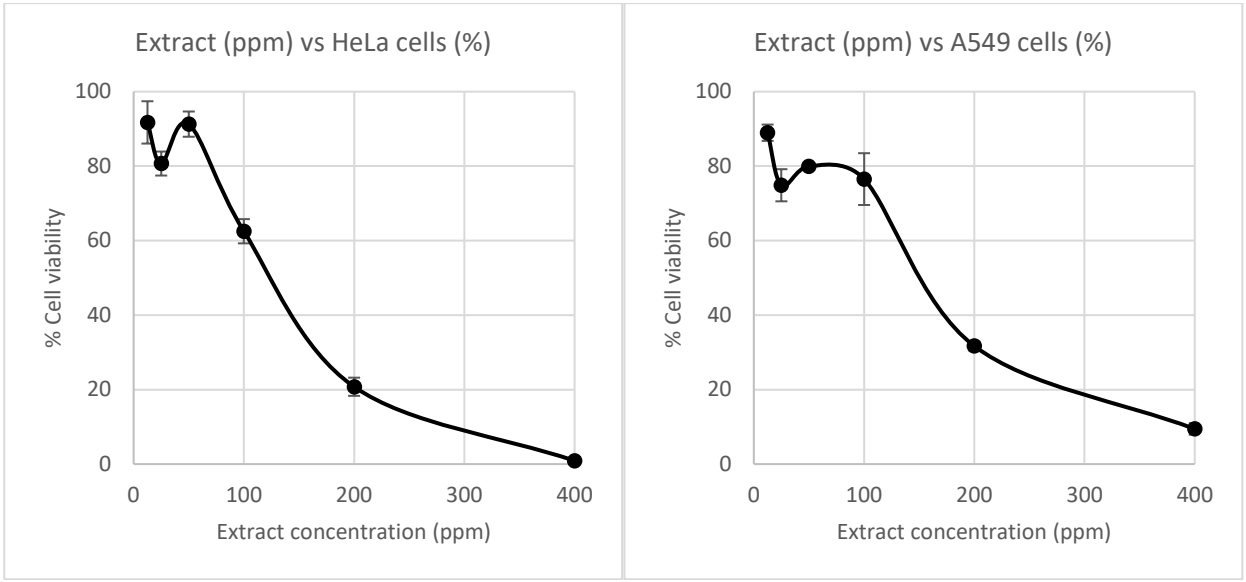
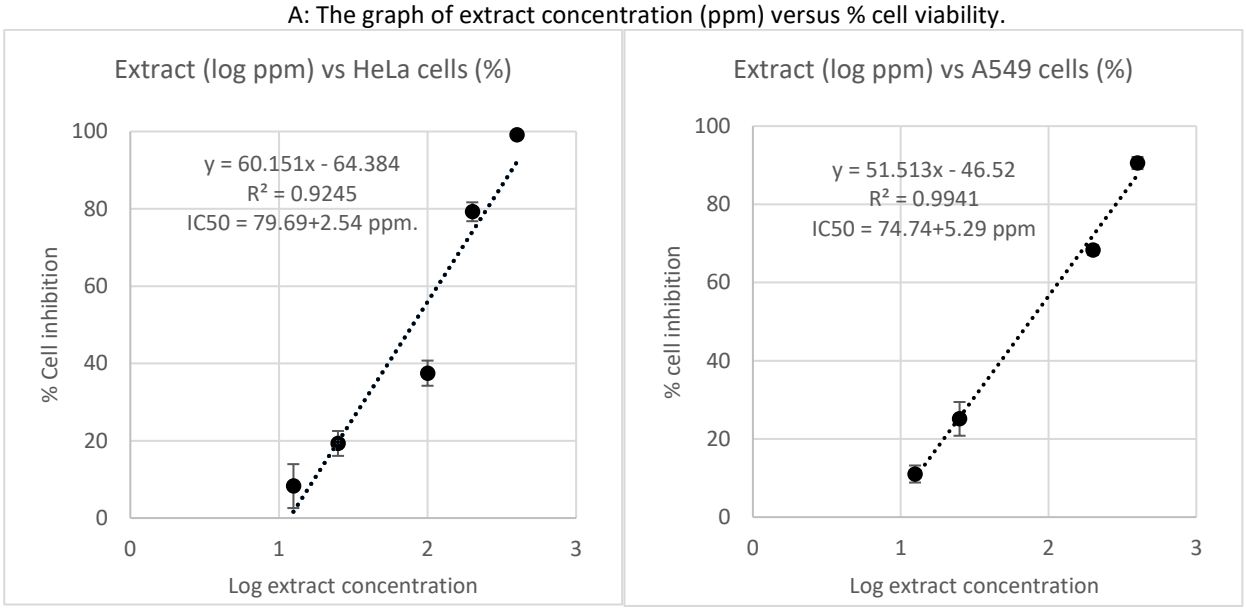


Figure 1: FTIR analysis of the ethyl acetate extract of *Cantigi* leaves



A: The graph of extract concentration (ppm) versus % cell viability.



B: The graph of log extract concentration versus % cell inhibition.

Figure 2: The graph of extract concentration (ppm) versus % cell viability (A) and the graph of log extract concentration versus % cell inhibition

Discussion

Cervical and lung cancer cases in 2018-2020 have increased worldwide and in Indonesia. Several causes underlie cervical cancer, including human papillomaviruses (HPV), smoking, and HIV infections (ACS, 2021b), while lung cancer may be caused by smoking tobacco, as the leading cause, secondhand smoke, exposure to other risk factors, such as radon and asbestos, in addition to genetic factors (ACS, 2021c).

The ethyl acetate extract of Cantigi leaves was standardised by evaluating the specific and nonspecific parameters as seen in Tables I and II. The parameters were analysed using the methods described in a book published by the Direktorat Jenderal Republik Indonesia. The results met the standards (Ditjen POM RI, 2000). The pH of the ethyl acetate extract was acidic (2.87) due to the acid components of the authors' unpublished research data, namely the results of the analysis of volatile compounds by GCMS (palmitic acid and stearic acid) and nonvolatile compounds by LCMS (trametenolic acid). These acidic compounds have anticancer activity, especially in breast cancer cells (Evans *et al.,* 2009; Zhang et al., 2014; Zafaryab, 2019). The FTIR analysis data showed the presence of functional groups O-H, C-H aliphatic, C=O, C-H, C=C, and N-H (Table II and Figure 1). The pH of the ethyl acetate

of Cantigi leaves, the three acids identified, and the FTIR analysis data may be related to the cytotoxic activity of the extracts on cancer cells.

Figure 2A shows the profile of the relationship between the extract concentration and the percentage of viability of cancer cells. Increasing the extract concentration caused a decrease in HeLa cancer cells and A549 lung cancer cells, with almost the same pattern of decline. The IC_{50} value of the ethyl acetate extract of Cantigi leaves for each cancer cell can be determined by changing the graph of the extract concentration versus the percentage of viability of cancer cells into a log of the extract concentration versus the percentage of inhibition of cancer cells (Figure 2B). The IC_{50} values of 74.74 ± 5.29 ppm in A549 cells and 79.69±2.54 ppm in HeLa cells reflect a potent cytotoxic activity. This paper is the first report on the cytotoxic activity of Cantigi leaf ethyl acetate extract on HeLa cervical cancer cells and A549 lung cancer cells.

Conclusion

The ethyl acetate extract of Cantigi leaves met specific and nonspecific parameters and showed potent in vitro cytotoxic activity on HeLa cervical cancer cells and A549 lung cancer cells.

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Conflicts of interest

The authors state that there is no conflict of the interest

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