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The Anticancer Activity of Srikaya Leaves Fraction (*Annona squamosa* L.): An In Vitro Study

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Abstract

Background

Anticancer drugs are aimed primarily at inhibiting the growth and proliferation of cancer cells. Srikaya leaves (*Annona squamosa* L.) had been proven to possess various therapeutic effects and potential to be developed as anticancer drugs due to its cytotoxic activity.

Objective

This study aimed to assess the anticancer activity of srikaya leaves (*Annona squamosa* L.) fraction.

Methods

Methanol fraction of srikaya leaves were obtained at concentrations of 500; 250; 125; 62.5; 31.25 µg/ml. Srikaya methanol fraction and cisplatin as control were given to a plate that was sealed with T47D cells for MTT assay. Identification of compounds in the methanol fraction of srikaya leaves was performed with thin layer chromatography (TLC). Data were collected in the form of absorbance value and half-maximal inhibitory concentration (IC50) value was determined by linear regression. Data analysis was carried out with paired T test, unpaired T test, and ANOVA.

Results

Average percentage of T47D cells viability increased with the decrease in the concentration of srikaya methanol fraction. Obtained IC50 value was 174.25 µg/ml which was quite active and potential to be developed as an anticancer drug. Methanol fraction of srikaya leaves contained secondary terpenoid metabolites, steroids, phenols, flavonoids, alkaloids and tannins. Flavonoid was the dominant metabolites in phytochemical tests and believed to play a major role in cytotoxic activity of srikaya leaves.

Conclusion

Methanol fraction of srikaya leaves possessed the cytotoxic effect on T47D cancer cell line through the role of flavonoid metabolites.

Keywords: srikaya, *Annona squamosa,* anticancer, T47D cells

Introduction

Cancer is of the many diseases prominent as the leading cause of burden and mortality in the world. According to World Health Organization (WHO) in 2018, the global cancer burden is

estimated to have risen to 18.1 million new cases and 9.6 million deaths. The morbidity is one in 5 men and one in 6 women, with a mortality of one in 8 men and one in 11 women. Worldwide, the 5-year prevalence or total survivor within 5 years is estimated to be 43.8 million [1].

Anticancer drugs are aimed primarily at inhibiting the growth and proliferation of cancer cells. Molecular targets for anticancer drugs in breast cancer cells include estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2), and vascular endothelial growth factor (VEGF). The target for induction of apoptosis and anti-apoptotic inhibition involves the p53-mitochondrial and TNF-related apoptosis-inducing ligand (TRAIL) receptors, nuclear 2 transcription factors, cell cycle processes, signal transduction and angiogenesis [2].

Srikaya (*Annona squamosa* L.) had been proven to possess various therapeutic effects [3-8] and potential to be developed as anticancer drugs [9]. This plant contains several active compounds including flavonoids, borneol, camphor, alkaloids, terpenes, saponins, tannins, polyphenols and polyketide compounds [10]. Some previous studies had shown that srikaya plants possessed potential cytotoxic activity [11]. This study was conducted to complement previous research and was carried out only at the cytotoxic test stage. This study was conducted to assess the efficacy of the polar fraction of srikaya leaves (*Annona squamosa* L.) on cytotoxic activity against T47D cells.

Methods

This study was an experimental in vitro study using the T47D breast cancer cell line. Srikaya leaves were obtained from the Traditional Medicine Research and Development Center, Tawangmangu, Indonesia. Simplicia of the srikaya leaves were first dried and mashed, then extracted with methanol solvent by maceration for 3x24 hours. Simplicia filtration and pulp maceration were performed. Macerate was evaporated with a rotary evaporator to obtain the thick extracts. The obtained extract was added by aquadest in 1:1 ratio. N-hexane solvent as much as 200 ml (5x200ml) was added and then separated with a separating funnel. Ethyl acetate solvent as much as 200 ml (5x200 ml) was added. The n-hexane, ethyl acetate and methanol fractions were obtained. In this study, the test fraction to be explored was methanol.

Methanol fraction from srikaya leaves was diluted with dimethyl sulfoxide (DMSO) and Dulbecco's modified Eagle's medium (DMEM) to obtain concentrations of 500; 250; 125; 62.5; and 31.25 µg/ml. Cisplatin was used with a concentration of 50: 25: 12,5: 6,25: 3,125 µg/ml. Furthermore, the test fraction and cisplatin were given to a plate that was sealed with T47D cells for the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Identification of compounds found in the methanol fraction of srikaya leaves was performed with thin layer chromatography (TLC).

Data were collected in the form of absorbance from enzyme-linked immunosorbent assay (ELISA) reader. From the absorbance values, cytotoxic activity of the test compounds was expressed by half-maximal inhibitory concentration (IC50) values which was determined with linear regression. The IC50 value indicated the concentration value which results in 50% inhibition of cell proliferation. The cytotoxic activity against cancer cells was classified as "very active" if the IC50 value was <10 µg/ml, "active" if the IC50 value was 10-100 µg/ml and "quite active" if the IC50 value was 100-500 µg/ml [12]. Data analysis was carried out with paired T test, unpaired T test, and ANOVA.

Results

Table 1. Efficacy of Srikaya Leaves Fraction against Cancer Cell Line Viability

No	Treatment	Cell Line Viability (%) Mean ± SD
1	MF 500	46.78±0,014*
2	MF 250	49.89±0,016*
3	MF 125	59.84±0,007*
4	MF 62.5	62.97±0,029*
5	MF 31.25	65.79±0,021*
6	CIS 50	23.60±0,070
7	CIS 25	38.13±0,021
8	CIS 12.5	56.10±0,032
9	CIS 6.25	61.60±0,070
10	CIS 3.125	63.19±0,035

MF: Methanol fraction; CIS: Cisplatin; * p< 0,05 vs Cis 3,125 *posthoc test*

As shown in Table 1, methanol fraction of srikaya leaves affected the viability of cancer cells. The highest concentration of 500 µg/ml resulted an average viability of 46.77% and the average percentage of viability increased with a decrease in the concentration of the test fraction at a concentration of 250 µg/ml (49.89%), 125 µg/ml (59.84%), 62.25 µg/ml (62.97%), 31.25 µg/ml (65.79%), respectively. The obtained IC50 value was 174.25 µg/ml. Based on the classification, the IC50 value of methanol fraction of srikaya leaves was quite active and potential to be developed as an anticancer drug.

As exhibited in Table 2, the methanol fraction of srikaya leaves contained secondary terpenoid metabolites, steroids, phenols, flavonoids, alkaloids and tannins. Flavonoid was the dominant metabolites in phytochemical tests and believed to play a major role in cytotoxic activity of srikaya leaves.

Tabel 2. Phytochemical Analysis of Srikaya

Fraction	Spot Colors	Metabolite
Methanol Fraction	Purple, yellow, dark yellow and brown	Terpenoid, steroid, phenol flavonoid, alkaloid and tannin

Discussion

Flavonoid compounds are known to possess the ability to induce apoptosis. Apoptosis as the programmed cell death plays an important role in the process of cancer regulation. The mechanism of flavonoid in inducing apoptosis is through the inhibition of DNA topoisomerase I/II activity, modulation of signaling pathways, decreased expression of Bcl-2 and Bcl-XL genes, increased expression of Bax, Bak and p53 genes, and activation of endonuclease [13, 14]. Flavonoids were the main compounds capable of spurring apoptosis with a variety of mechanisms in the methanol extract of kenikir leaves (*Cosmos caudatus* Kunth) which had cytotoxic properties against T47D cells with IC50 of 344.91 µg/ml. A study showed apoptotic death of myeloma cancer cells due to the influence of the chloroform fraction of papaya leaves (*Carica papaya* L.) with the main content of alkaloids allegedly through the initial stages of inhibiting the enzyme of DNA Topoisomerase II. With the inhibition of DNA topoisomerase enzyme activity, the process of the bonding between the enzyme and the DNA of the cancer cell was prolonged, protein linked DNA

breaks (PLDB) was formed, resulted in DNA fragmentation or damage to cancer cells and subsequently affected the cancer cell replication process [15-18].

Conclusion

Methanol fraction of srikaya leaves possessed the cytotoxic effect on T47D cancer cell line through the role of flavonoid metabolites.

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