The effect of soursop leaves

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The Effect of Soursop Leaves Fraction (*Annona squamosa* L.) as Anticancer

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A B S T R A C T

Anticancer drugs are primarily aimed at inhibiting the growth and proliferation of cancer cells. The soursop plant (*Annona squamosa* L.) has the potential to be developed as an anticancer drug. This plant contains several active compounds including flavonoids, borneol, camphor, alkaloids, terpenes, saponins, tannins, polyphenols, and polyketide compounds. This study was conducted to assess the efficacy of the polar fraction of soursop leaves on cytotoxic activity based on the IC_{50} value in T47D cells. This research is experimental in vitro study using cell line T47D. The methanol fraction of soursop leaves was diluted with DMSO and DMEM to obtain a concentration of 500; 250; 125; 62.5; 31.25 µg/ mL cisplatin with a concentration of 50:25:12,5: 6,25:3,125 µg/mL. The methanol fraction of soursop from the highest concentration of 500 µg/ml has average viability of 46.77% and the average percentage of viability will increase in proportion to the decrease in the concentration of the test compound. The IC50 value shows the concentration value that results in the inhibition of cell proliferation by 50% of the population. In conclusion, the methanol fraction of soursop leaves have an anticytotoxic effect on the T47D cell line through the role of flavonoid metabolites.

1. Introduction

Cancer is a disease that causes misery and death in humans. According to WHO data in 2013, the incidence of cancer increased from 12.7 million cases in 2008 to 14.1 million cases in 2012. Meanwhile, the number of deaths increased from 7.6 million people in 2008 to 8.2 million in 2012. Cancer is the second-largest mortality cause in the world at 13% after cardiovascular disease. It is estimated that in 2030 the incidence of cancer can reach 26 million people, and 17 million of them die from cancer, especially in poor and developing countries, the incidence will be faster.[1-4]

Anticancer drugs are primarily aimed at inhibiting the growth and proliferation of cancer cells. Molecular targets for anticancer drugs on breast cancer cells include estrogen receptors (ER), HER2 (Human epidermal growth factor receptor 2), and vascular endothelial growth factor (VEG). The targets for apoptosis induction and antiapoptosis inhibition include the p53-mitochondrial pathway and the TRAIL receptor, nuclear two transcription factor, cell cycle process, signal transduction, and angiogenesis.[5-7]

The soursop plant (*Annona squamosa* L.) has the potential to be developed as an anticancer drug. This plant contains several active compounds including flavonoids, borneol, camphor, alkaloids, terpenes, saponins, tannins, polyphenols, and polyketide compounds. Several previous studies have shown that soursop plants have cytotoxic activity. Magadula et al. (2009) stated that the methanol extract of soursop leaves had a toxic effect on Artemia salina larvae with

an LC50 value of 0.64 µg/ mL. The cytotoxicity test of 32 methanol extract and chloroform fraction of Annona squamosa L. leaves on HeLa cells gave LC_{50} values of 4.5467 and 7.6984 µg/mL, respectively.[8-10]

Previous research found that the cytotoxic activity of the polar fraction and extract of soursop leaves (*Annona squamosa* L.) against T47D had a cytotoxic activity value of T47D cells with an IC_{50} value of 110.3 µg/mL. This study was conducted to complement previous studies and was carried out only at the cytotoxic test stage. This study was conducted to assess the efficacy of the polar fraction of soursop leaves (*Annona squamosa* L.) on cytotoxic activity based on the IC_{50} value in T47D cells.

2. Methods

This research is experimental in vitro study using cell line T47D. Soursop plants were obtained from the Research and Development Center for Traditional Medicine, Tawangmangu, Indonesia. Simplisia of soursop leaves first dried and mashed, then extracted with methanol solvent by maceration 3 x 24 hours. Then filtering of the simplicia and macerate dregs is carried out. The macerate was evaporated using a rotary evaporator to obtain a thick extract. The extract obtained was added with a 1: 1 comparative aqua dest, added 200 ml (5x200ml) of n-hexane solvent then separated with a separating funnel then added 200 ml (5x200 ml) ethyl acetate solvent, to obtain the fractions of n-hexane, ethyl acetate, and methanol. In this study, the fraction to be tested is the methanol fraction.

The methanol fraction of soursop leaves (*Annona squamosa* L.) was diluted with DMSO and DMEM to obtain a concentration of 500; 250; 125; 62.5; 31.25 µg/ mL cisplatin with a concentration of 50: 25: 12,5: 6,25: 3,125 µg/mL. Next, the test fraction and cisplatin were given to a plate filled with T47D cells for MTT assay. Identification of the compounds contained in the methanol fraction of soursop leaves using the TLC (Thin Layer Chromatography) test. The data collected was in the form of absorbance data that was read from the ELISA reader. The absorbance value can then determine the IC50 value with linear regression.

Data analysis was carried out by homogeneity test: Lavene test was then carried out before after each group with paired T-test then continued, before-after test using unpaired T-test then continued with ANOVA test and dose suitability performed post hoc.

3. Results and Discussion

Table 1 shows that the methanol fraction of soursop (*Annona squamosa* L.) from the highest concentration of 500 µg/ml has average viability of 46.77% and the average percentage of viability will increase in proportion to the decrease in the concentration of the test compound, namely 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%) with IC50 values of 174.25 µg/ mL. The cytotoxic activity of the test compound was expressed by IC50, which was determined by linear regression. The IC50 value shows the concentration value that results in the inhibition of cell proliferation by 50% of the population. The classification of the extract's cytotoxic activity against cancer cells can be classified as very active if the IC50 value is <10 µg/mL, the active category if the IC50 value is 10-100 µg/mL, and the category as quite active if the IC50 value is 100-500 µg/ mL. Based on this classification, the water-methanol fraction of soursop leaves (*Annona squamosa* L.) has an IC50 activity of 174.25 µg/mL which is quite active and has the potential to be developed as an anticancer drug.

Table 2 shows that the methanol fraction of soursop leaves contain secondary metabolites of terpenoids, steroids, phenols, flavonoids, alkaloids, and tannins. Flavonoids are the primary metabolites that are more dominant in phytochemical tests. This metabolite is believed to play a significant role in the ability of the methanol fraction of soursop leaves as an anticytotoxic.

Flavonoid compounds are known to induce apoptosis. Apoptosis is programmed cell death and plays a vital role in the process of cancer development. The mechanism of flavonoids in inducing apoptosis is

through 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 endonuclease activation. Research by CCRC (2008), shows that flavonoids are the main compounds capable of stimulating apoptosis with various mechanisms in the methanolic extract of Kenikir leaves (Cosmos caudatus Kunth) which has cytotoxic properties against T47D cells with an IC50 of 344.91 µg/ml.[11-15]

Studies show that myeloma cancer cell death by apoptosis is due to the influence of the chloroform fraction of papaya leaves (*Carica papaya* L.) with the main content of alkaloids, it is thought that through the initial stages of inhibiting the DNA Topoisomerase II enzyme. By inhibiting the activity of the DNA Topoisomerase enzyme, the process of binding between the enzyme and the DNA of cancer cells is getting longer, so that a Protein Linked DNA Breaks (PLDB) will be formed. As a result, there is fragmentation or damage to the DNA of cancer cells which subsequently affects the replication process of cancer cells.

Table 1. Viability cell line after treatment with methanol fraction of soursop (*Annona squamosa* L)

No	Treatment	Mean of Viability Cell Line (%) ± SD
1	FMA 500	46.78 ± 0.014*
2	FMA 250	49.89 ± 0.016*
3	FMA 125	59.84 ± 0.007*
4	FAM 62.5	62.97 ± 0.029*
5	FAM 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

FMA: Methanol Fraction; CIS: Cisplatin; * p< 0,05 VS Cis 3,125 post hoc test

Table 2. Phytochemical analysis of soursop

	Spot Colour	Metabolite
Methanol Fraction	purple, yellow, dark yellow, and brown	Terpenoid, steroid, fenol flavonoid, alkaloid and tannin

4. Conclusion

Methanol fraction of soursop leaves has an anticytotoxic effect on the T47D cell line through the role of flavonoid metabolites.

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