

## PHYTOCHEMICAL SCREENING AND ANTI-BREASTCANCER ACTIVITIES OF ANNONA MURICATA (L.) LEAF EXTRACTS

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### Abstract

The objective of the research is to study antiproliferation activities (anti-breastcancer) of leaf extracts soursop (*Annona muricata* L.) from five locations growth in West Java, Indonesia against breast-cancer cell line Michigan Cancer Foundation-7 (MCF-7). Each of the soursop leaf from five locations, was extracted with 70% ethanol using a microwave as a tool for extraction. In vitro test against breast cancer cells growth was conducted using the MCF-7 cell line by means of the MTT-assay.. Based on the analysis of phytochemicals, all soursop leaf extracts contain alkaloids, triterpenoids, tannins, saponins and flavonoids, but did not contain any steroids compound. Soursop leaf extracts with the highest anticancer activity were derived from Sukabumi 1 with an IC<sub>50</sub> value of 12.5 µg/ml. The results obtained from this study indicate the ability of soursop leaf extracts to inhibit the growth of breast cancer cells (MCF-7). Such extracts could be used in the treatment of cancer patients.

**Keywords:** *Annona muricata* L., anti-breastcancer, MCF-7, soursop leaf

## Introduction

Cancer is a major global killer, especially in developed countries, and it has become the second killer in developing countries [1]. Cancer is known as a dreaded disease due to its intractable nature and causes many deaths. Breast cancer is influenced by the effects of estrogen, the androgen cycle, estrogen use, and carcinogens such as smoking, ultraviolet radiation, unbalanced diets and stress. Based on data from the Hospital Information System (SIRS) in 2010 [2], breast cancer is ranked highest for the number of patients treated in Indonesia (28%), followed by cervical cancer (12%).

In the early stages, breast cancer forms bumps on mammary tissue and at a later stage there will be lesions to cuts in the mammary tissue. To treat cancer of the affected cells are subjected to radiation therapy and chemotherapy. Radiation therapy and chemotherapy are aim at destroying the cancer cells and control the growth of tumors in the mammary tissue, and control the growth of tumors caused by 'secondaries' arising from the initial cancer.

Presently research is concentrated on cancer drugs, to treat cancer and reduce the adverse effects of chemotherapy and increase the chance of patient survival. The use of drugs such as doxorubicin is one way to cope with the development of cancer and reduce the formation of secondary cancers. However, the adverse effects of treatment with doxorubicin are difficult to cope with such as hair loss, a changed heartbeat, and a decreased white blood cell count which is an agent of the body's defenses. This condition is a challenge in primary cancer treatment. One of the challenges in cancer treatment is to improve the effectiveness and reduce the side effects of the main treatment. Effort is therefore needed to discover dan develop drugs based on natural ingredients (herb). One of the alternatives is the chemical components in soursop leaf. The advantage of this is that soursop leaf contains antimicrobial [3], wound healing [4], apoptosis inducing [5], chemo-beneficiated [6], antioxidant [7] and others pharmacological compounds [8]. Soursop leaf extracts can exhibit significant which does not affect normally growing cells. The objective of the research is to study the

anti-proliferation activities of ethanol extracts from five locations of soursop leaf in West Java, Indonesia, using the MCF-7 cancer cell line.

## Methods

### Materials

Soursop leaf was collected from two locations in Subang and three locations in Sukabumi, West Java, Indonesia, and cancer cells MCF-7 from the collection Agency for the Assessment and Application of Technology, Indonesia.

### Procedure

This study involved sample preparation, extraction of soursop leaf, phytochemicals analysis of extracts, creation of a work culture, observation of effects of the number of samples tested for MCF-7 cell population, or testing the effect of adding the sample to the cell viability of MCF-7 cell line at inhibitive concentrations.

### Sample Preparation

Soursop leaf were harvested and sub dried. Once dry, crushed soursop leaf were prepared. Ready for extraction.

### Sample Extraction

Soursop leaf extraction process was a modified method derived from various authors [9,10,11,12]. The extraction process used a microwave oven.

### Phytochemical Analysis

Qualitative testing of soursop leaf extract includes analysis for the presence of alkaloids, flavonoids, terpenoids, steroids, saponins and tannins [13].

**Alkaloids Test.** A total of 1.0 mg of soursop leaf extracts is inserted in a test tube, then we added two drops of ammonia and 5 ml of chloroform and the mixture filtered. The filtrate was added to 1.0 ml 2.0 M  $H_2SO_4$ . The existence of alkaloids in soursop leaf extract is indicated by the formation of a red precipitate.

**Triterpenoids and Steroids Testing.** A total of 1.0 mg of soursop leaf extract was added to a test tube, then add about 5 ml of hot ethanol was added and the mixture filtered. The filtrate was evaporated

after which we added 1 ml of di-ethyl ether. After shaking with 'vortex mixer', we added 1.0 ml of concentrated  $H_2SO_4$  and 1 ml of  $CH_3COOH$ . The formation of red or gray color indicated the presence of tri-terpenoids and a green color indicated the presence of steroids in the soursop leaf extract.

**Flavonoids Test.** A total of 1.0 mg of soursop leaf extract is inserted into a test tube and 5.00 ml of water added, and the mixture filtered. The filtrate obtained was combined with Mg powder, 1.0 ml of concentrated HCl, and 1.0 ml iso-amyl alcohol. The mixture is then stirred to give layers. Colors formed between the interface with iso-amyl alcohol indicate the present of flavonoids.

**Tannins Test.** A total of 1.0 mg soursop leaf extract is inserted into a test tube, then we added 5.0 ml of water and the mixture filtered. To the filtrate are added 3 drops of 1%  $FeCl_3$ . The formation of blue or blackish green indicates the soursop leaf extract contains tannins.

**Saponins Test.** A total of 1.0 mg soursop leaf extract is inserted into a test tube and to this we added 5.0 ml of water and then filtered the mixture. The filtrate was shaken and allowed to stand for 10 min. The formation of a stable froth showed saponin was present in soursop leaf extract.

#### **Cytotoxicity test against Breast Cancer Cells**

Antiproliferation activity was tested in cell culture with the MCF-7 cancer test using Thiazolyl Blue Tetrazolium Bromide assay (MTT-assay) [9]. Doxorubicin is used as a positive control and DMSO solution as a negative control. Condensed extract is dissolved in solvent DMSO to make stock solutions of 10 %. The stock solution is then diluted in RPMI 1640 medium to make solution substock at 1% (v/v). Dilution tests are carried out in order to obtain a final concentration of multilevel test solution of 250, 100, 50, 10, and 1.0  $\mu g/ml$ . Then 20 ml of test solution of various concentrations is added to the plate wells which already contain cancer cells, and then incubated for 24 h at a temperature of 37 °C in 5.0 % (v/v)  $CO_2$ . After the addition of the test solution back we incubated the cells for 24 h. The number of living cells per well was calculated using an ELISA Reader at a wavelength of 570 nm.  $IC_{50}$  values are determined from the graph of percent living cells against the concentration of the test sample. The percentage of MCF-7 cell-death of each

test solution is computed using the formula: % Cell death =  $(Ab-Au)/Ab \times 100$  %. Description: Ab : Absorption blank (DMSO) and Au : Absorption test solution.

#### **Results**

The content of chemical compounds from every location is different, especially in the number, qualitatively evident from the appearance of chemical compounds contained in extracts of soursop leaf (Table 1). The compounds detected in soursop leaf extracts were saponins, alkaloids, tannin and flavonoids, but no soursop leaf extracts contained steroid compounds. It is apparent there is no positive reaction from the steroids as there was no green color in the test solution. Figure 1 shows the existence of a positive reaction (+) based on qualitative analysis of flavonoids in soursop leaf extracts. The qualitative phytochemical as shown in Figure 1 are summarized in Table 1.

The extract of soursop leaf origin from Sukabumi I show the most active with  $IC_{50}$  at 12.5  $\mu g/ml$ , followed by Subang II. Graphical  $IC_{50}$  presentations can be seen in Figure 2. Conditions MCF-7 cancer cells due to administration of the ethanol extract of the soursop leaf is shown in Figure 3. Here the breast cancer cells MCF-7 suffered severe damage and cause debris at an extract concentration of 250  $\mu g/ml$ . Most of soursop leaf extracts have the ability close to equal to the concentration of doxorubicin at 0.5  $\mu g/ml$  in inhibiting breast cancer cells MCF-7 *in vitro*. The state of breast cancer cells MCF-7 as result of various extracts of soursop leaf is presented in Figure 4.

#### **Discussion**

The absence of steroid compounds in soursop leaf extract is different from other research (3) who found steroid compounds in soursop leaf phytochemical analysis from Abuja, Africa, but the same result with the other researchers in reference [7]. Flavonoids and alkaloid in soursop leaf are like compounds in propolis [9, 15]. Figure 1 shows the existence of a positive reaction (+) based on qualitative analysis of flavonoids in soursop leaf extracts.

From Table 2 indicates that the extract of soursop leaf from Sukabumi I is very active in

inhibiting the growth of breast cancer cells MCF-7, showing higher activity in comparison with other soursop leaf extracts from different locations and positive control doxorubicin. This shows that sampling location is very important [9].

Differences in anticancer material in leaf extracts may be caused by the difference in acetogenin substances. Piperine from the Piper group includes amide alkaloids are active components in inhibiting cancer [16, 17]. Other compound in soursop leaf which are in anticancer effects are from the class of alkaloids, such as piplartin. The content of sterols also will affect the role of inhibition of cancer cell [18]. The content of piperine or piplartin will be different due to the influence of the location such as climate and soil which supports the growth of soursop leaf

From Figure 4 it is apparent that the soursop leaf extracts at a concentration of 250 µg/ml showed significant cell death even causing the destruction of cancer cells. Inhibition of cancer cells from the extract of this soursop leaf was in accordance to other research [5, 6]. Likewise, when compared with the effect on cancer cells T47D [19], IC<sub>50</sub> values of soursop leaf extracts are higher. Secondary metabolites used as anticancer compounds include phenol groups, one of which is the acetogenins [20, 21, 22]. According to [23] there are five forms monotetrahydrofuran in acetogenins which act as an active anticancer component. In addition [24] found that acetogenins compounds in soursop leaf can serve as an anticancer agent. Opinion [25] says that the soursop leaf can be used in treating cancer and to prevent cancer cell growth. In addition, soursop leaf has been tested in treating cancer in rats induced by carcinogenic materials of plant origin from *Cycas* sp.

We performed the extraction of soursop leaf. Based on the analysis of phytochemicals, all soursop leaf extract contains alkaloids, triterpenoids, tannins, saponins and flavonoids, but did not contain a steroids. Soursop leaf extract with the highest anticancer activity were derived from Sukabumi I West Java, Indonesia with IC<sub>50</sub> value of 12.5 µg/ml. All Soursop leaf extract can inhibit cancer cells MCF-7 at a concentration of 250 µg/ml. The results obtained from this study indicate the ability of soursop leaf extract to inhibiting the

growth of breast cancer cells (MCF-7). This is a potential treatment for cancer patients.

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No	Locations	Chemical Compounds							
		Alkaloids			Triterpenoids	Steroids	Flavonoids	Tanins	Saponins
Dragendorf	Meyer	Wegner							
1	Sukabumi I	+++	++	+++	+	-	+	+++	+++
2	Sukabumi II	+++	+++	+++	+++	-	++	+	++
3	Sukabumi III	+++	+++	+++	+++	-	+++	++	+++
4	Subang I	+++	+++	+++	++	-	+++	+++	++
5	Subang II	+++	++	+++	++	-	+++	+++	++

Informations : - = not detected, + = fair detected, ++ = good detected, +++ = very good detected, ++++=very very detected

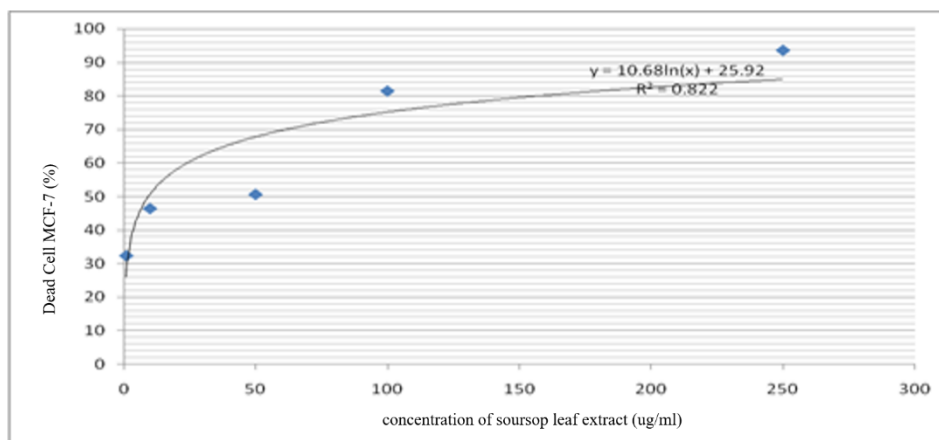
**Table 1** The results of the qualitative phytochemical analysis of soursop leaf extracts

No.	Locations	Value of IC <sub>50</sub> MCF-7
1.	Sukabumi I	12.5
2.	Sukabumi II	102.5
3.	Sukabumi III	42.5
4.	Subang I	57.5
5.	Subang II	17.5
6.	Doxorubicin 0.5 µg /ml	58.79

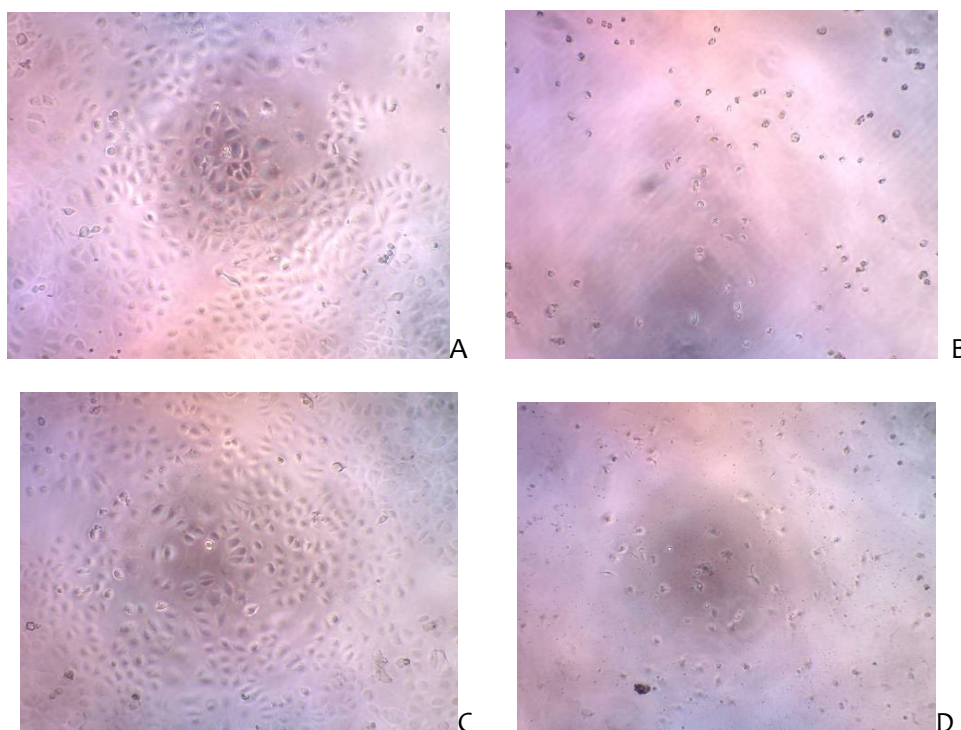
**Table 2** Original Soursop leaf extracts and IC<sub>50</sub> values MCF-7 cancer cells due to treatment soursop leaf extracts



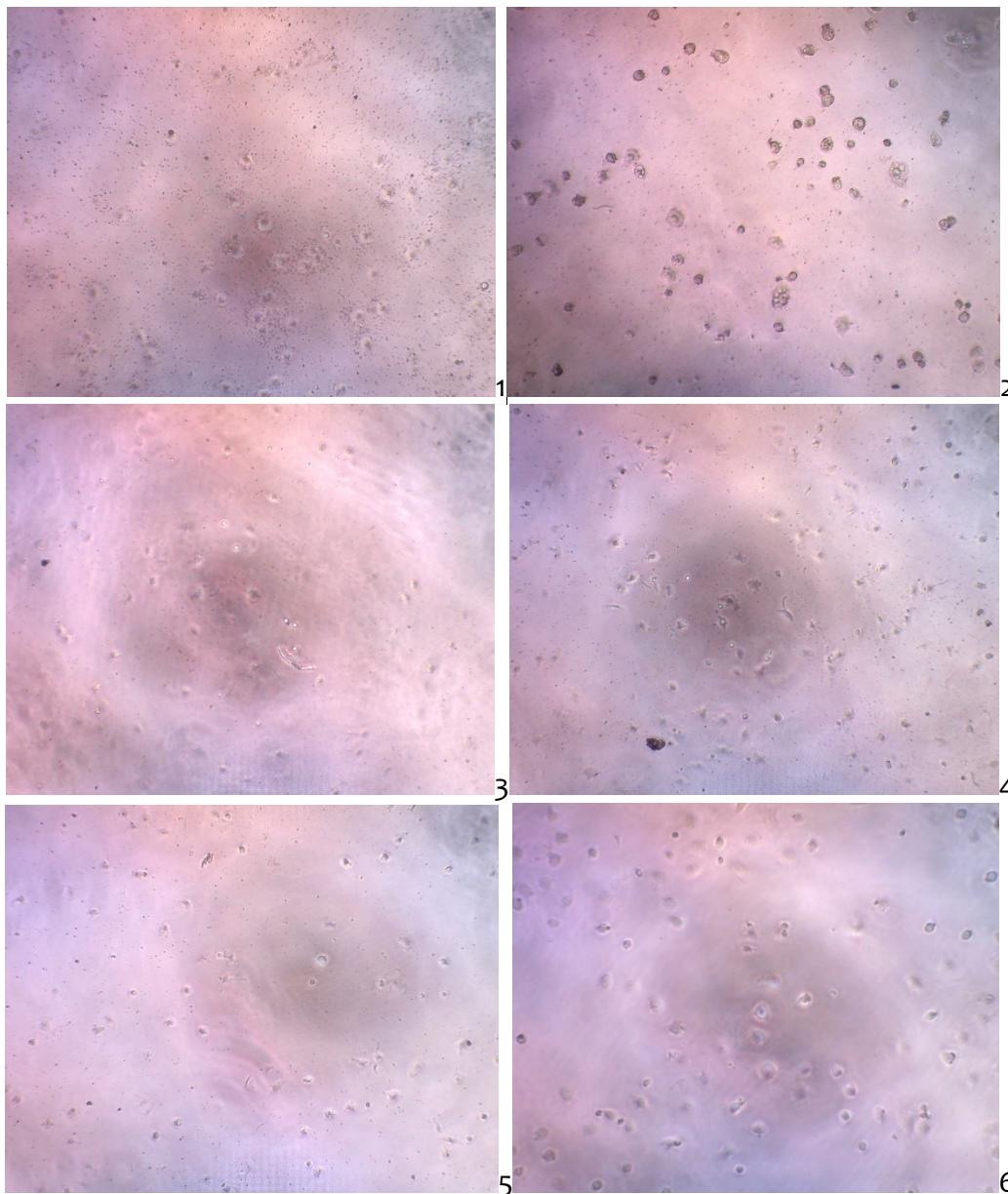
**Figure 1.** A positive result of flavonoids compounds in the test solution



**Figure 2:** Graphic of IC<sub>50</sub> calculation influence extract soursop leaf



**Figure 3:** Conditions of breast cancer cells MCF-7 as a result of extract soursop leaf Description : a=control no treatment, b=doxorubicin 0.5 µg /ml, c =concentration 1 µg /ml, and d = concentration 100 µg /ml



**Figure 4:** Conditions of breast cancer cells MCF-7 due soursop leaf extracts at concentration of 250 µg/ml. Description: 1-5=Soursop leaf extract treatment results in accordance with the location of the origin of soursop leaf, 6=the effect of doxorubicin 0.5 µg /ml