Effect of Soursop Leaf Extract Tablets (*Annona muricata* L.) against Cancer Cells

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ABSTRACT--- ***Among the many plants that grow in Indonesia, which can be used for alternative medicine, one of them is the soursop (Annona muricata L.). Acetogenin derived from the soursop plants touted able to overcome the 12 cancer cells. In America, a sample from Indonesia tested on of lung cancer cells, breast, colon, and a variety of other cancer cells.***

Making oral dosage form in tablet soursop leaf extract can produce a more practical preparation when compared to the stew. Fraction of ethyl acetate soursop leaf tablet was tested in vitro for anticancer activity against several cancer cell-derived cell (cell line) such as HeLa cells (cervical), MCF 7 (breast) and A549 (lung).

The results obtained from tablet formula still does not requirements of disintegration time. Therefore, it is necessary to re-formulation with the addition of the disintegration to a maximum of 20%. Cytotoxic test results of soursop leaf extract tablets (Annona muricata L.) against cervical cancer cells (HeLa), breast cells (MCF-7) and lung cells (A549) has been shown to kill cancer cells specifically. From the test results of the research, soursop leaf extract (Annona muricata L.) IC_{50} above 30 ppm, is not classified as active as anticancer. However, the IC_{50} value against HeLa cell extracts (33.98 ppm) and the breast cells (32.90 ppm) approaching the threshold for active categorized.

Keywords--- anticancer, pharmaceuticals, cytotoxic

1. INTRODUCTION

Soursop (*Annona muricata* L.) is a plant that is believed to kill cancer cells, treatment of gallstones, antisembelit, uric acid and increase appetite. The National Cancer Institute found acetogenin active compounds in soursop leaves (Annona muricata L.) which can resist a variety of cancer cells. Other studies have found that the soursop has antitumor and anticancer effects are very strong. Soursop active ingredient has a strong effect in slowing the growth of cancer cells. According to The Journal of Natural Products, one of the chemical elements that annonaceous acetogenin contained in soursop able to choose, distinguish, and kill cancer cells that develop in the colon. Efficacy acetogenin in crippling cancer cells continue to be studied and researched by scientists from various countries (Artini, 2012).

Utilization of natural materials, especially natural vegetation is mostly in the form of a traditional drug classes such as herbs, while in pharmaceutical dosage forms such as tablets, caplets and capsules are still very rare. So, people are accustomed to eating soursop leaves in the form of decoction. It is expected to manufacture oral dosage in tablet form from the fraction of ethyl acetate extract of soursop leaves can produce a more practical preparation when compared to the stew. Tablets proved to be very beneficial, because it is easily stored and carried practical and appropriate dose adjustment. Tablets are the ingredients in a solid dosage form that is usually made by adding additional appropriate pharmaceutical (Elisya, 2013).

This research work is in continuation of previous study, so the material extracted from the ethyl acetate fraction obtained from the results of the research. The results of the ethyl acetate extract fraction tested by LCMS (Liquid Chromatography-Mass Spectrometry) obtained acetogenin compound; murihexocin-A, annomuricin-E, muricapentocin, gigantetrocin, corossolon. Levels of total acetogenin the ethyl acetate fraction of soursop leaves (Annona muricata L.) is ranged from 5.570 to 5.941%. Extracts from ethyl acetate fraction of the soursop leaf is toxic to HeLa cells (<1000 ppm), and has potential as anticancer (Elisya, 2013).

Tablets extracts from ethyl acetate fraction soursop leaf is made with a dose of 100 mg, then formulated using wet granulation method. Selection of wet granulation method because of the nature acetogenin relatively stable in the presence of moisture and to obtain a good flowability to be easily compressed. From previous studies, with doses above 100 mg tablets resulted in poor quality with large weights, so that the tablet is made with a dose of 100 mg to fix the formula, so that the resulting tablet can meet the requirements of the fourth edition of the Pharmacopoeia Indonesia.

In this research we have tested the different effect of the tablet of ethyl acetate extract prepared from leaves soursop (Annona muricata L.) by wet granulation method against several cancer cells using cell lineages (cell line) as HeLa cells (cervical), MCF-7 (breast) and A549 (lung) *in vitro*.

Based on the previous by Junaedi, et al, 2013, reported that the hypoglycemic activity of herbal extracts Roots cat (Acalypha indica L.) differ significantly with extracts in tablet dosage forms, therefore in this study once it is made tablet formulation it was necessary to test the activity.

2. METHODS

This research is an experimental research (true experimental) in the field of pharmaceutical and pharmacological which require ethical clearance. The study was conducted in the Department of Pharmacy at the Polytechnic of Jakarta II and Primate Studies Center, Institute for Research and Community Service Institute of Agriculture (IPB) in August - October 2014.

a. Ethyl acetate extract fraction soursop leaf (Elisya, 2013)

Ethyl acetate extract fraction obtained from the leaves of the soursop plants that grow in the Bekasi area, Indonesia. Soursop leaves washed, dried with aerated, then pulverized in a blender. Weigh number (10 kg) soursop leaf (Annona muricata L.) that has been refined and incorporated into the container maceration, 80 liters of ethanol 96% was added to soak the whole crude drug. This mix was left in the maceration container for several days (5 days) and then the aqueous extract obtained was concentrated using a rotary evaporator, and then into the oven temperature of 50 ° C to obtain a thick extract.

Viscous extract obtained partitioned by adding a number of extracts into the solvent (ethanol: n-hexane) at a ratio of 1: 1, then add distilled water with the same ratio and mix using an electric mixer until a few moments. After mixing is complete, the obtained two layers of immiscible, the water layer is separated by a solvent and then the fraction of water with a new solvent (ethyl acetate). The process is complete when the solvent used has been clear.

b. MTT *assay* .

MTT assay test is one method used in the cytotoxic test. Cytotoxic test is used to determine the IC_{50} value parameter. IC_{50} value indicates the concentration that resulted in inhibition of cell proliferation by 50% and demonstrate the potential toxicity of a compound to sel. This value is a benchmark to test cell kinetics observation.

The principle of the method of MTT is a yellow tetrazolium salt reduction of MTT (3- (4,5-dimetiltiazol-2-yl) -2.5-difeniltetrazolium bromide) reductase system. Succinate tetrazolium included in the respiratory chain in the mitochondria of living cells to form purple formazan crystals and not water soluble. Reagent addition stopper (is detergenik) will dissolve these colored crystals which are then measured absorbance using an ELISA reader. Purple color intensity is proportional to the number of living cells. So if the greater intensity of the color purple, the mean number of living cells more (Dewi, 2012).

Procedure:

1. Plate cells (104 – 106 cells) in 200 ml PBS in 96-well (flat bottom).
2. Add 20 ml of MTT solution, mix well.
3. Incubate for 4h in 37C in dark.
4. Remove aliquot for analysis; add 200 ml acidic isopropanol and mix well.
5. Incubate additional 1h in 37C in dark.
6. Read plate in ELISA Reader – measure OD in 570 nm (background wavelength is 630nm).

c. Tablets formulation

The tablets formulation was developed based on a formula tablets consisting of filler, binder, and lubricant. Each auxiliary materials are added to the appropriate concentration.

Formulation : Soursop leaf fraction : 100 mg; Avicel pH 101: Lactose (1: 1) : qs; Corn starch : 10%; PVP 6 – 14%; LHPC-LH_{11}: 5 – 10 %; Magnesium stearate: 1%; Talc 1%; Aerosol : 0,5%

Each raw material is weighed according to the formula. Mixing is done by mixing the viscous extract with a filler in the wet granulation process with PVP binder. Dry the granules in the oven. Sift the dry granules and add external charger, stirring until homogeneous.

Make checks printed mass includes examining test: moisture content, flow properties, and kompressibilitas. When it meets the requirements, perform the checks printed tablets and tablets that include test: visual test, uniformity of weight, uniformity of size, hardness, crispness, disintegration time and test activities.

3. RESULTS and DISCUSSION

Table 1. Results of testing the tablet mass.

No.	Testing	F1	F2	F3	F4	F5	F6	requirement
1	Levels of moist	4,0	3,2	2,9	3,6	4,0	3,3	2-5%
2	The nature of the flow	2,2	2,2	2,2	2,0	1,6	1,6	≤ 2,5 sec
3	Compressibility	11,22	15,00	11,00	10,00	11,22	10,00	≤ 20%

In making the above formula gives the violence that does not qualify hardness below 4 kg / cm2, so the re-formulation of the addition of PVP binder 12-14%.
With the increase in the addition of a binder, produce tablets which meet the requirements, with the violence over 4 kg / cm2. But the disintegration time of tablets to be more than 15 minutes, so do come back formulation with the addition of external crusher LHPC-LH_{11} much as 10%.

Table 2. Results of testing the tablet.

No.	Testing	F1	F2	F3	F4	F5	F6	requirement
1	uniformity of size	0,52<1,53< 1.17	0,52<1,16< 1,18	0,53<1,15< 1,20	0,56<1,16< 1,26	0,56<1,15< 1,26	0,56<1,16< 1,26	4/3t<D<3 t
2	uniformity of weight	0,21 - 3,27	0,19 - 3,57	0,06 - 4,71	0,22 - 2,18	0,09 - 3,45	0,18 - 3,46	<5%
3	violence	3,20	3,44	3,77	5,29	6,57	4,33	≥ 4 kg/cm^2
4	crispness	0,38	0,78	0,32	0,80	0,26	0,00	< 1%
5	disintegration time	9'	11'	12'	25' 57"	> 35'	> 25'	≤ 15 minute

Tablet disintegration time is the time required for the destruction of the tablet in a suitable medium, so that no part of the tablet is left on the screen. Factors that affect: the physical properties of the granules, hardness, porosity and absorption granule tablet. Increase of pressure at the time of the process causes a decrease in porosity and increase the hardness of tablets. Due to the increase in tablet hardness will inhibit the penetration of liquid into the pores of the tablet, thereby extending the tablet disintegration time.

The addition of binder produces hardness of tablets that meet the requirements but produces a disintegration time which does not meet the requirements of more than 15 minutes, so that a re-formulation with the addition of external crusher LHPC-LH11 as much as 10%. However, the results obtained tablet formula still does not meet the requirements of disintegration time is still passing 15 minutes. Therefore, it is necessary to re-formulation with the addition of the crusher to a maximum of 20% (Siregar, 2007).

For tablet in accordance with the quality requirements in terms of tablet disintegration time, is necessary to re-extract formulation with the addition of soursop leaf shredder inside.

Table 3. Results of MTT test.

No.	Cancer Cell	IC_{50} (ppm)
1.	A549	54,66
2.	HeLa	33,98
3.	MCF-7	32,90

Cytotoxic test results soursop leaf extract tablets (Annona muricata L.) against cervical cancer cells (HeLa), breast cells (MCF-7) and lung cells (A549) has been shown to kill cancer cells specifically. An extract is considered active if it is able to inhibit the growth of 50% of the population of cancer cells / tumor at concentrations below 30 ppm (IC_{50} <30 ppm) (Marraskuranto, 2008). From the test results of the research, soursop leaf extract (Annona muricata L.) IC_{50} above 30 ppm, is not classified as active as anticancer. However, the IC_{50} value against HeLa cell extracts (33.98 ppm) and the breast cells (32.90 ppm) approaching the threshold for active categorized.

4. CONCLUSIONS

a. Soursop leaf extract tablet formulation (Annona muricata L.) produce tablets that still does not meet the requirements of disintegration time according to the fourth edition of the Pharmacopoeia Indonesia.
b. IC_{50} value against HeLa cell extracts (33.98 ppm) and the breast cells (32.90 ppm) approaching the threshold for active categorized.

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