Cytotoxic Effects of Cassava (*Manihot esculenta* Crantz), Adira-2, Karikil and Sao Pedro Petro Varieties against P-388 Murine Leukemia Cells

Widiastuti Diana[1]*, Mulyati Ade Heri[1], Eka Herlina[1], Siti Warnasih[1], Suchyadi Yudhie, Triastinurmiatiningsih[2] and Supratman Unang[3]
1. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor, INDONESIA
2. Department of Biology, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor, INDONESIA
3. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, INDONESIA
*dianawidi25@unpak.ac.id

Abstract
This research examines the cytotoxic effect of cassava (Manihot esculenta Crantzs*) in inhibiting growth of P-388 murine leukemia cancer cells. This study uses three cultivar of cassava samples from West Java Indonesia including Adira-2, from Cimahpar Bogor, Karikil from Cililin West Bandung and Sao Pedro Petro from Cisarua, Bogor. Cassava was first extracted with ethanol at room temperature, which was then partitioned succesively with n-hexane, ethyl acetate and n-butanol.*

All of the extracts were evaluated against P-388 murine leukemia cells in-vitro using MTT assay. As a result, the n-hexane extract of San Pedro Petro cassava from Cisarua Bogor has an inhibition concentration (IC_{50}) value of 15.8µg/mL which can prevent the growth of murine leukemia P-388 cancer cells.

Keywords: Cassava, Cytotoxic, Adira-2, Karikil, *Sao Pedro Petro,* P-388 murine leukemia cell.

Introduction

In 1962, Dr. M Goenawan, the head of RSTP (Lung Tuberculosis Hospital) in Cisarua, Bogor, started treating cancer using 10-30 grams raw cassava (*Manihot esculenta* Crantz) per day, chewed up gradually to allow the medicinal substances and enzymes to be absorbed by oral cavity mucosa. Besides eating raw cassava, the patients were also given compression of grated cassava, especially in the areas of cancer and parts of body with lymph glands (neck, armpit, stomach and thigh crease).

A survey on the total cyanogen content of cassava roots and products from the cassava growing in Indonesia had been carried out involving thirty samples of cassava starch and other specialised products which had a mean cyanogen content of only 5 µg/mL whereas 29 samples of cassava flour, chips and gaplek gave a much higher mean cyanogen content of 54 µg/mL[1].

Cyanogenic Potential of roots is the highest in the one closer to the cortex (550 µg/mL), while only 35 µg/mL in the central part of root[2]. Bradbury et al[3] suggested sampling 10 mm thickness of roots in the central parts of the roots and taking diagonal part of the cross vertical section.

Cassava varieties locally used as medicines are used as samples in this research. They are *Manihot esculenta* Crantz Adira 2 planted in Cimahpar, Bogor, Karikil from Cililin West Bandung and *Sao Pedro Petro* cassava from Cisarua, Bogor.

Leukemia was first described by Virchow in 1847 as "white blood". It is a cancer that attacks the blood cells in the bone marrows. Under normal conditions, white blood cells will develop regularly when the body needs them to eradicate emerging infections. However, in leukemic patients, their bone marrows will produce abnormal and excessive white blood cells. The excessive amounts will accumulate in the bone marrows, reducing the number of healthy blood cells.

The extraction was performed with an ethanol solvent which was then partitioned successively with *n*-hexane, ethyl acetate and *n*-butanol. The cytotoxic test used the method of Alley et al[4].

Material and Methods

Location: This research was carried out in the Chemical Laboratory of Universitas Pakuan, Laboratory of Natural Chemistry of Universitas Padjajaran for the extraction and partition and the Institut Teknologi Bandung for the cytotoxic tests.

Materials and Tools: Rotary evaporator, blender, ethanol, *n*-hexane, ethyl acetate, *n*-butanol, distilled water, acetone and general glassware used in the laboratory.

Plant Material: Samples used in this research were tubers of Adira 2 cassava planted in Cimahpar, Bogor district, *Sao Pedro Petro* cassava was planted in Cisarua, Bogor district and Karikil cassava was planted in Cililin West Bandung district.

Extraction: Cassava was peeled, washed and weighed as much as 200 grams and then blendered until it was smooth. 96% ethanol solution was added to it and then macerated for 24 hours. This was repeated 3 times. The filtrate was evaporated using a rotary evaporator at the speed of 55 rpm and temperature of 50°C. From the evaporation, an ethanol extract was obtained and put into vial I. Vial II, in the

meanwhile, was added with 50 mL water and then homogenized. Next, the solution was put into a separating funnel and added with 100 mL *n*-hexane and extracted for 15 minutes and repeated two times. The *n*-hexane phases were accommodated in a boiling flask and evaporated. The remaining *n*-hexane extract was put into vial II.

In the aqueous phase, 100 mL ethyl acetate solution was added and extracted for 15 minutes and repeated two times. In the phase of separated ethyl acetate, it was placed in a boiling flask and evaporated. The remaining ethyl acetate extract was put in vial III. In the aqueous phase, 100-mL solution of *n*-butanol was added and then extracted for 15 minutes and repeated two times. The *n*-butanol phase was collected in a boiling flask and evaporated and the remaining *n*-butanol extract was put in vial IV. In a separated water phase, it was put in a boiling flask and evaporated and the remaining extract was put in vial V.

Determination of IC_{50} Value of Murine Leukemia P-388 Cancer Cells: The cytotoxic test of cancer by MTT method (3-(4, 5-dimethylazol-2-yl)-2, 5-diphenyltetrazolium bromide) was done as follows: cancer cells with a concentration of 3×10^3 cells / 100 µL were distributed into wells and incubated for 24 hours in a carbon dioxide incubator, to enable them to adapt and stick to the wells. Then, 100 µL culture medium containing ethanol extract sample (vial I) was added to each well and re-incubated for 48 hours. At the end of the incubation, the culture medium containing ethanol extract sample (vial I) was discarded and washed with 100 µL PBS (Phosphate Buffered Saline).

Next, into each well, 100 µL culture medium containing MTT was added and incubated for 4 hours at 37°C. Living cells were expected to react to MTT and form purple formazans. After 4 hours, a stopper reagent was added to each well to kill the cells and dissolve the formazan crystals. The plate was shaken using a shaker for 10 minutes, then incubated at room temperature in a dark room overnight. Next, the absorbance of each well was determined using an ELISA reader at a wavelength of 595 nm. Experiment was carried out for samples with *n*-hexane extract, *n*-butanol, ethyl acetate and water extracts.

Results and Discussion

The three samples are demonstrated in figure 1. Adira 2 varieties are known for their oval leaves resembling fat fingers, with purple tips and red stalks and light green at the bottom. Its tubers have brownish white outers and purple in the inside and are bitter in taste. Sao Pedro Pedro cassava, originated from Brazil has slightly greenish or brownish foliages with 7-9 strands and smaller in size. The upper leaves are dark red and pink at the bottom. The stems are small, long and low branched. The tubers are not stemmed, crawling and poking out.

Extraction: The extraction method used in this research was maceration which is a sample immersion using organic solvents at room temperature. This process is very advantageous in isolating natural material compounds because soaking will break walls and cell membranes due to the differences in pressure between the internal and external environment of the cells.

The secondary metabolites in the cytoplasm will dissolve in organic solvents, resulting in perfect extraction. The stillness in the maceration process decreases the active material movements. Stirring can balance the concentration of the ingredients in the liquid too while speeding up contacts among samples. The selection of solvents for the maceration process will be determined by the solubility of the natural compounds in the solvent[5].

Varieties/ Local Names	Leaves	Tubers
Adira-2		
Karikil		
Sao Pedro Petro (SPP)		

Figure 1: Adira-2, Karikil and *San Pedro Petro* cassava

The organic solvent used in this study was 70% ethanol due to it being polar, as the active compounds in the cassava tubers are generally polar too. The process of maceration of a material is influenced by the duration of soaking. The longer is the soaking, the greater is the chance to withdraw the phytochemical contents thus increasing the result up to the saturation point of the solution. In this research, the sampling time was 3×24 hours. Contacts between the samples and the solvent can be increased by stirring so that contacts occur more frequently, perfecting the extraction process.

The extract was filtered to separate the residue and filtrate. Then, the obtained filtrate was separated from its solvent using a vacuum rotary evaporator at 50°C as at this temperature, the secondary metabolite contents of cassava tubers were expected not to be denatured by the heat treatment which could be too high. The evaporation produced a viscous extract. The extraction results in ethanol, *n*-hexane, butanol, ethyl acetate and water solvents used for cytotoxic tests of murine leukemia P 388 cells are shown in table 1.

From the inhibition percentages obtained from each extract, the water fraction gave the greatest inhibition, characterized by the smallest IC_{50} among all fractions. S1 and S2 samples in all fractions provided considerable inhibition above 30 µg / Ml, thus resulting in the S1 sample from Cimahpar and S2 originating from Sukaraja in all solvent fractions to be considered as inactive. S3 cassava sample in alcohol fraction, ethyl acetate, *n*-butanol and water had an inhibition above 30 µg / mL. Thus, its cytotoxic criterion was not active. But, in *n*-Hexane solvent, it had 15.847 µg / mL inhibition. Thus, it was categorized medium cytotoxic, according to Alley et al[4].

Table 1
Cytotoxic Tests on Murine Leukemia P-388 Cell IC_{50}

	IC_{50} (µg/mL)		
Extracts	**Adira-2**	**Karikil**	***Sao Pedro Petro (SPP)***
Alcohol	58.497	90.311	61.218
n-Hexane	77.956	57.587	15.847
Ethyl Acetate	85.758	85.355	39.900
n-Butanol	73.544	50.166	50.245
Water	42.886	57.317	58.770

Based on the results of the above cytotoxic test on the murine leukemia P 388 cells, it is shown that cassava tubers from Cimahpar and Sukaraja have inactive cytotoxicity while those originating from Cisarua with *n*-Hexane extract have medium cytotoxicity.

Conclusion

In-vitro cytotoxic activity aganist murine leukemia P-388 cell line at cassava (*Manihot esculenta Crantz*) with 3 different varieties were evaluated. Cytotoxic effect against P-388 leukemia cancer cell line is considered as a predictive anticancer activity indicator and IC_{50} value of Cassava *Sao Pedro Petro (SPP)* variety from Cisarua Bogor district of Indonesia was 15.847 µg/mL which indicates that Cassava *Sao Pedro Petro (SPP)* hexanolic extract is potentially present as an interesting cytotoxic activity and should be evaluated against primary cuturers to determine the selectivity of their effect.

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