International Maize Conference
Gorontalo, Indonesia, November 21-23, 2012

Agribusiness of Maize-Livestock Integration

Ministry of Agriculture
in collaboration with
Provincial Government of Gorontalo

The Sorghum Functional Foods Utilization to Prevent Cancer in Foods Diversification Efforts

Yuszda K. Salimi

Department of Chemistry, State University of Gorontalo

ABSTRACT. Sorghum is potential source of antioxidants because it contains phytochemical components such as flavonoids, phenols hydroquinone, sterols and tannins. Phenolic antioxidants and flavonoids in sorghum as scavenger to free radicals potentially inhibit cancer cell proliferation. This research aims to study the activity of extracts of hexane, ethyl acetate, and ethanol extracts of sorghum on the inhibition of proliferation of several cancer cell line. All three extracts of sorghum were evaluated on the cytotoxic *Artemia salina* Leach, lymphocytes, lung cancer cells (A549), colon cancer cells (HCT 116), cervical cancer cells (Hela), and lymphoma cancer cells (Raji). The results showed sorghum extract can inhibit proliferation of cancer cells. The concentration of extract in inhibiting cancer cell varied and generally the higher the concentration the higher the inhibitory activity of extracts of cancer cells. Sorghum extract inhibits proliferation of A 549 cancer cells d" 24%, HCT 116 d" 22%, Hela d" 25%, and Raji cancer cells d" 80%. Sorghum extract has the highest inhibitory activity on Raji cells. Inhibition of cancer cells Raji (80.08%) were shown by ethanol extracts of sorghum (2600ig/ml).

Key words: sorghum extracts, cancer cell lines, lymphocyte, A 549, HCT 116, Hela, Raji

Introduction

The relationship between foods and health that have often been researched and it has been proven can stimulate the functional food products that positively affect health. Sorghum is potential to be developed in Gorontalo because it can grow up in dry area and contains complex carbohydrates and food fibers which potentially prevent cancer. Phytochemical components in sorghum extract consist of flavonoid, phenol hydroquinone, sterols and tannins. Those chemical components have different solubility based on solvent polarity level and are more concentrated to ethyl acetate and ethanol extract. The total concentration of phenol in ethyl acetate > ethanol > hexane (Salimi 2012). The higher phenol concentration in sorghum the higher it seizes free radicals. The presence of natural antioxidant prevents oxidative damage and diseases which wrap the free radicals reactions in. (Amic *et al.* 2003)

Cell culture has been the important tool in molecular and cellular biological research; it well model system to physiology and biochemical cells research; the effect of food components toward cell, mutagenesis and carcinogenesis. Cell line is often used in cancer research by consideration psycho-chemical can be controlled and cell physiological condition can be constantly maintained. Cells directly access material or tested matters, so that the needs of tasted sample are relatively small. Lymphocyte cell line is often used as a model to know the effect of food materials toward human immune system. Lymphocyte cell culture is not quite different with other cell line culture systems. Lymphocyte cell does not stick on the glass or plastic surfaces as in mono layer cell culture (Frashney 1994).

So that, cell culture testing by *in vitro* is important to be conducted to know the sorghum extract potency toward lymphocyte cells and cancer cells.

Materials and Methods

Kawali variety sorghum are gained from farmers at Kidul Mountain Area. Lung cancer cells A549 (ATCC CCL-185) the collections of FKH IPB Tissue Culture Laboratory, Cervix cancer cells HeLa (ATCC CCL-2), lymphoma cancer cells (Raji) (ATCC CCL-86), and colon cancer cells HCT-116 (ATCC CCL-247) from Stem Cell Cancer Institute Jakarta.

Cytotoxic Testing towards *Artemia Salina L* Larva by using *Brine Shrimp Lethality Test* Method (Meyer *et al.* 1982)

Brine Shrimp Lethality Test (BSLT) is a toxicity testing to *Artemia Salina L* larva. This test is done in hexane extract, ethyl acetate, and ethanol. Biological testing is conducted by putting ten (10) 48 hours Artemia Salina larva in a bottle that filled of extract solution and sea water with required concentrate. The observation is done after 24 hours. The number of dead larva is written than counted,

the next data is analyzed by using prohibit analysis, the SAS 640 computer program to investigate the LC50 data.

Cell Suspension and Cancer Cell Culture Preparation

A549 cell, HCT 116, HeLa and Raji are maintained in a DMEM/F12 (*Dulbecco's Modified Eagle Medium)* culture medium which equipped by 10% *fetal bovine serum* (FBS), 100U/ml penicillin and 100ug/ml streptomycin 1%. The cells are incubated in 37°C, 5% CO_2.

Cell culture is transferred in 96 well plates, each plate 1000 ¼À1 and 3 empty plates as medium control. The condition of cells is observed by using inverted microscope to observe cell distribution. The cells have to be incubated in one night. The treatment for sample cells will be done after the cells are in a normal condition. Then, concentration series without treatment (cell control and DMSO control) and sorghum powder extract with ½ time, once, 1½ time and two times LC_{50} for treatment. The plates which have been filled by cells are moved out from CO_2 incubator. For monolayer cell (A549, HeLa and HCT 116) cell medium is casted out (the plat is overturned to 180°). Doxorubicin is the positive control of anti-cancer. The cell culture is incubated in 5% CO_2, 37°C and 90% RH incubator.

At the end of incubation period, there is a testing by using MTT method to measure the proliferation inhibition activity. Tetrazolium Salt Reagan (MTT) is added in each micro plate, and then it is incubated during 4 hours. The cell cancer inhibition activity testing is measured by using elisa reader in at λ 595 nm absorption with 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.

The absorption data treatment is measured and converted in a formula as follow:

$$\% \text{ inhibition} = (1\text{-}OD_{sample}/OD_{control}) \times 100\%$$

OD: Optical Density (Absorption) at λ595 nm.

RESULTS AND DISCUSSION

Sorghum Extract Cytotoxic towards Artemia Salina Leach

Cytotoxic testing is a first testing which is done in observing pharmacology effect of one chemical compound.

The data that shown in table 1 is mortality data that is analyzed by using Probit Analysis Method to get LC_{50} values (Lethal Concentration 50%). The data shows the cytotoxic LC_{50} value of Hexane Extract < 1000 µg/ml and ethyl acetate extract & ethanol > 1000 µg/ml. LC_{50} is used as concentration or dosage in sorghum extract testing of cancer cell.

The Sorghum Extract Potency in Proliferation Cancer Cell

Sorghum extracts bioactivity testing towards of cancer cell by using MTT Method. The sorghum extract inhibition potency is tested at HeLa cancer cell line, A549, and HCT 116. A549 cell line is from lung carcinoma cell of 58 years man with morphology that resemble epithelial. HCT 116 cell line is the inherited cell of colon cancer of human being

Table 1 The Result of BSLT Testing of the Three Kinds of Extracts

Kinds of extract	Concentration (µg/ml)	Mortality presentation (%)		LC_{50} (µg/ml)	
		S0	S50	S0	S50
Hexane Extract	10	35.8	43.3		
	100	41.2	45.7	151	198
	1000	100	95.1		
	5000	100	100		
Ethyl Acetate Extract	10	34.1	15.3		
	100	32.8	31.7	1224	1352
	1000	42.2	25.5		
	5000	100	100		
Ethanol	10	17.4	12.9		
	100	21.2	10.6	2679	2595
	1000	39.6	43.8		
	5000	100	55.1		

S0 = Sorghum seed extract unpolished
S50 = Sorghum Extract DS 50%

(late phase adenocarcinoma). HeLa cell is inherited from human cervix cancer epithelia cell. These three kinds of cells morphologically is epithelial cell which has polygonal shape and it sticks on the monolayer cell. The Raji cell is the cell which come from lymphoblastoid cell culture that inherited from lymphoma burkitt. Burkitt is a kind of cancer which found in lymph system specifically in Lymphocyte B. this kind of cell includes in lymphoblast cell which morphologically round shape and it grows in suspension without stick on the surface.

The data in figure 1 showed that the anti-proliferation towards the cancer cell line is found in the three groups of extracts. The kind of solvent influences the proliferation inhibition. The inhibition of HeLa cervix cancer cell by sorghum ethyl acetate extract > ethanol extract > Hexane extract. The higher inhibition value is 25.4% at 2440 µg/ml concentration of unpolished sorghum ethyl acetate extract. The ethyl acetate solvent can extract alkaloid, aglycon and glycoside, sterols, terpenoid and flavonoid (Houghton & Raman 1998; Cowan 1999).

Proliferation inhibition of HCT 116 cancer cell by sorghum ethanol extract > ethyl acetate extract > hexane extract. The higher inhibition value is 22.3% at 5200 µg/ml concentration sorghum extract DS 50%. The proliferation inhibition of A549 cancer cell by sorghum ethanol extract > ethyl acetate extract > hexane extract. The higher inhibition value is 23.7% at 4020 µg/ml unpolished sorghum extract (figure 1). Hexane extract less shown the inhibition effect in HeLa cancer cell. It is assumed that the bioactive components which extracted in a low value of hexane solvent.

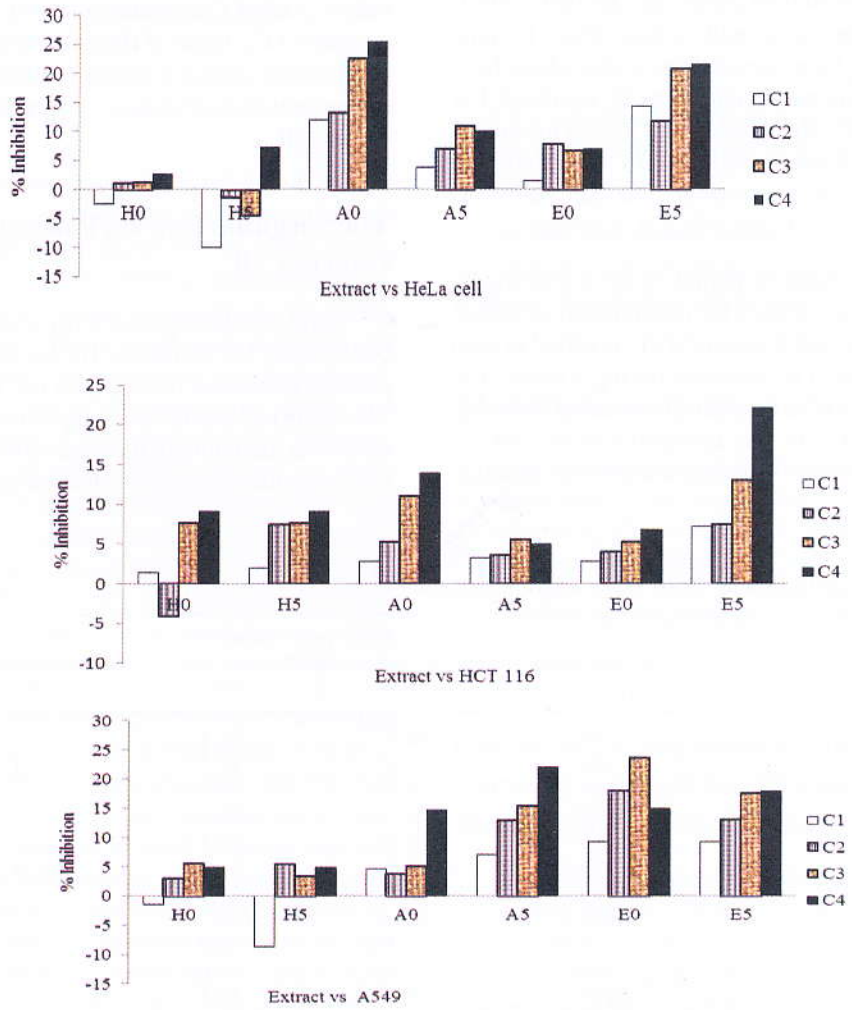

Figure 1. The effect of sorghum extract towards the proliferation inhibition HeLa cancer cell, HCT 116, A549. (H0 = hexane extract, A0 = ethyl acetate extract, E0 = Ethanol Extract, C1 = concentration ½ x LC_{50}, C2 = LC_{50} concentration, C3 = concentration x LC_{50}, C4 = concentration 2 xLC_{50}).

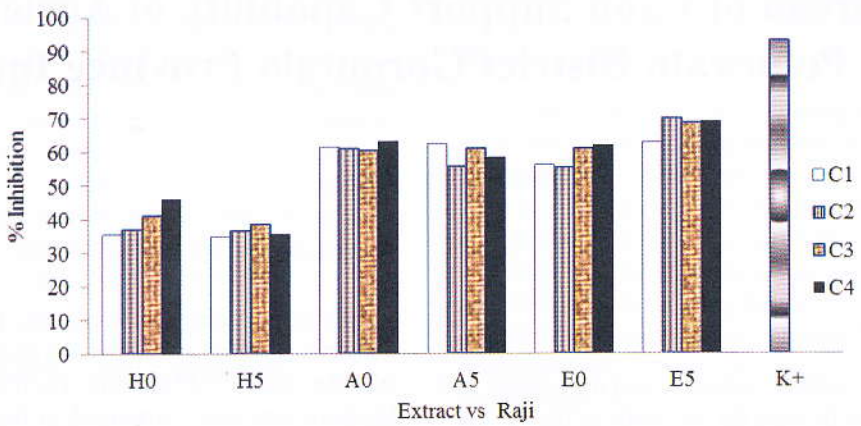

Figure 2. The effect of sorghum extract towards Raji cancer cell inhibition (H0 = Hexane Extract, A0 = Ethyl Acetate Extract, E0 = Ethanol Extract, C1 = concentration ½ x LC_{50}, C2 = concentration LC_{50}, C3 = concentration 1½ x LC_{50}, C4 = concentration 2 x LC_{50}).

The three extracts as explain above inhibit Raji cancer cell proliferation that comes from cell line lymphoblastoid which descended from lymphoma Burkitt (figure 2). Raji cancer cell inhibition (80.08%) is found in sorghum ethanol extract (2600µg/ml). The crude extract of several sorghum varieties inhibited cancer cell proliferation with concentration IC_{50} 59-389 2600 µg/ml in HT-29 colon cancer cell and 98-604 µg/ml in esophagus cancer cell (Awika *et al.* 2009).

Cancer cell inhibition is presumably caused by flavonoid and anthocyanin involved in sorghum extract that affect arrest cell cycle, so that the cell could not proliferate. The AGS Gastric cancer cell inhibition mechanism by anthocyanin components (malvidin, sianidin, delfinidin, pelargonidin, peonidin) reported by Shih *et al.* (2005). Malvidin (0-200 µM) showed cell anti-proliferation activity and caused arrest cell cycle at G0/G1 level. Malvidin accumulation at G1 phase AGS cell 20% (100 µM) and 30% (200 µM). Cancer cells in proliferative cycle are sensitive cells towards the effect anti-tumor compound. Basically, cytotoxic compound works by ruining enzymes or substrates associated with DNA synthetic. Thus, bioactive compound involved in sorghum inhibit cells that are splitting or DNA synthetic.

Conclusion

Ethyl acetate and ethanol extract showed valuable and effective proceeds in inhibiting the growth and proliferation Raji lymphoma cancer cell 50% than A549, HCT 116, HeLa cancer cell. The cancer cell anti-proliferation activity by sorghum extract is presumably caused by sorghum polyphenol that affect arrest cell cycle that the cell cannot proliferate.

References

Amic, D., D. Davidovic, D. Beslo, and N. Trinajsti. 2003. Structure-radical scavenging activity relationships of flavonoids. Croatica Chem Acta 76: 55- 61.

Awika, J.M., L. Yang, and D. Jimmy. 2009. Comparative antioxidant, antiproliferative and phase II enzyme inducing potential of sorghum (*Sorghum bicolor*) varieties. J. Food Sci. 42:1041–1046.

Cowan, M.M. 1999. Plants product as antimicrobial agents. Clin Microb Rev. 4: 564-582.

Freshney, R.I. 1994. Animal cell culture: A practical approach. Oxford Washington DC: IRL Press.

Houghton, P.J. and A. Raman. 1998. Laboratary handbook for the fractionation of natural extracts. New York: Chapman & Hall.

Meyer, B.N., *et al.* 1982. Brine Shrimp : A convenient general bioassay for active plant constituent. Plant Res Medica 45: 31-34.

Salimi, Y.K. 2012. Peranan ekstrak dan tepung sorgum (*Sorghum Bicolor* L) dalam penghambatan kanker secara in vitro dan in vivo pada Mencit BALB/c [disertasi]. Bogor: Sekolah Pascasarjana, Institut Pertanian Bogor.

Shih, P.H., C.T. Yeh, and G.C. Yen. 2005. Effects of anthocyanidin on the inhibition of proliferation and induction of apoptosis in human gastric adenocarcinoma cells. Food Chem Toxicol 43: 1557–1566.