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Effect of Gamma Irradiation on Jati Belanda (*Guazuma ulmifolia* L) Against Human Cancer Cell Lines.

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Abstract. The herbal plants commonly used in Indonesia are Jati belanda (*Guazuma ulmifolia* L) which is commonly used as a medicine for high blood pressure and cancer. During the storage, to reduce contamination from bacteria, fungi, and other microorganisms can be used gamma irradiation. Gamma Irradiation Co-60 can be used as an alternative for preserving herbal plants. This study aimed to examine the effect of Co-60 (0 and 7.5 kGy) gamma-irradiation on *G. ulmifolia* plants on anti-cancer line properties (THP-1, K-562, and MCF-7). *G.ulmifolia* herb without irradiation (0 kGy) and gamma-irradiated 7.5 kGy using Co-60. The samples were macerated using ethanol as much as 2 replications. Cytotoxic tests were performed on ethanol extracts against line cancer cells (THP-1, K-562, and MCF-7) with varying concentrations of 5, 10, 20, 40, and 80 µg / ml. The results showed that irradiation doses of 0 kGy and 7.5 kGy were very active in inhibiting K-562 cancer cells with IC_{50} respectively 10.94 µg/ml and 16.74 µg/ml. Gamma irradiation can be used as an alternative in preserving medicinal plants without reducing the anti-cancer properties from the *G.ulmifolia*.

INTRODUCTION

The development of traditional medicines according to WHO data in 2016 has been increasing lately, the use of herbal medicines is used as a complement to primary treatment (1). In Indonesia, the use of herbal medicine or traditional herbal medicine is 49.53% and the Indonesian population who consumes herbal medicine as much as 95.6% feels the benefits after taking an herbal medicine (2). Increased use of herbal medicines due to safe with fewer side effects compared to the use of modern medicine (3).

One of the herbs used in Indonesia is Jati belanda (*Guazuma ulmifolia L*). *G ulmifolia* is widely used for traditional medicine as anti-bacterial, high blood pressure, and also cancer (4). The main content of *G ulmifolia* is a flavonoid, the flavonoid is secondary metabolites that exist in every plant that function to ward off free radicals through the donor H atom with OH groups in flavonoid. Free radicals in the human body can affect immunity, one of the diseases that is affected by immunity is cancer (5).

Cancer treatment using herbal plants is widely used, including in Indonesia. About 70-95% in developing countries use herbal medicines as additives in the treatment of cancer. The use of herbal plants is greatly influenced by the quality of the plants themselves, one of which affects the quality of herbal plants is that there is still a lot of contamination during the production and storage process before being used by consumers (6). Contamination of herbal plants can include fungi and bacteria that can cause problems that are dangerous for consumers who consume herbal plants that have been contaminated (1).

The gamma irradiation can be an alternative in reducing the contamination of herbal plants during the storage process so that it can be used as an alternative for preserving herbal plants. The preservation method uses gamma irradiation with no heat during the process and does not produce side products (7). The use of gamma irradiation for preservation purposes has been approved by the World Health Organization (WHO) and a dose of 30 kGy is still safe to use for plant material (8).

Research on the use of gamma irradiation has been carried out. Determination of flavonoid and total phenolic of suruhan herb to be irradiated gamma with a dose of 10 kGy did not have significant differences with controls

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(without irradiation) (6). Research on *G. ulmifolia* has been widely studied and efficacious as an anti-cancer, but the effect of gamma irradiation on *G. ulmifolia* as an anti-cancer has never been studied. For this reason, further research needs to be carried out on the effect of gamma irradiation on *G.ulmifolia* after gamma irradiation and their properties on cancer cells line.

MATERIALS AND METHODS

Extract Preparation

The dry herb of *G.ulmifolia* (200 grams) was wrapped using polyethylene plastic and irradiated using C0-60. The irradiation dose used was 0 (without radiation = control) and 7.5 kGy. Each radiation dose consists of 2 packages of *G. ulmifolia* (200 grams) that have been irradiated using Co-60. Samples were macerated using ethanol as much as 3L, maceration was carried out with 5 replications of maceration. The filtrate obtained was then evaporated using a Buchi rotary evaporator to obtain ethanol extract from *G.ulmifolia.*

Cytotoxicity assay

The cytotoxic activity of ethanol extract on cell line (THP-1, K-562, and MCF-7) was purchased from the University of Agriculture (IPB) and using variations concentration of 5, 10, 20, 40, and 80 µg / ml. Cells were ready for use with the final concentration is 2 x 105, after that was tested on a 24 well plate using ethanol extract with several variations of concentration using DMEM as a media cells and calf bovine serum 10% as a supplement for cell cancer. The well plate was incubated using a 5% CO2 incubator for 72 hours and calculated using a microscope at 4000x magnification using trypan blue staining [9].

Analysis data

IC_{50} data on ethanol extracts against cells line (THP-1, K-562, and MCF-7) were obtained from the conversion of live cell data to the calculation of % inhibition. The number of dead cells is obtained from the linear regression between log concentrations used and the probit of dead cells. The value of x is the log of concentration and y is probit, the IC_{50} value is obtained by determining the value of x on the probit with the value of axis is 5 by assuming the dead cell is 50% (9).

RESULT AND DISCUCCION

The moisture content of *G.ulmifolia* was 7.87%. The water content obtained is still following BPOM regulations which require the quality of medicinal plants to have water content below 10% (10). The yield of maceration using ethanol of 0 (without radiation) and 7.5 kGy obtained almost the same extract weight. An increase of 1.29% in the extract dose of 7.5 kGy due to the degradation of some components that have high molecular weight so that it will affect the solubility of the component (11). Prakash (2017) in his research revealed that higher irradiation will be increasing the extract. At doses of 10 and 25, kGy can increase the weight of extract about 5-30% compared to non-irradiated (11). The yield data of *G. ulmifolia* ethanol extract can be seen in table 1.

TABLE 1. Weight of ethanol extract from *G.ulmifolia*

Irradiation dose (kGy)	Color	Weigh of extract (gram)	Yield (%)
0	green	40.25	20.13
7.5	green	42.84	21.42

A plant can be said to be an anti-cancer drug if cytotoxic assay has been done on the plant. Cytotoxic properties of cancer cells can be used as a reference if the medicinal plant is used as a new anti-cancer drug from natural ingredients.

The result from the Cytotoxic activity of ethanol extract *G ulmifolia* showed that the higher the concentration would be inversely proportional to the number of living cells (Figure 1). The dose-dependent phenomenon is seen in Figure 1 curve, the percentage increase in cell inhibition along with the higher concentration given (12).

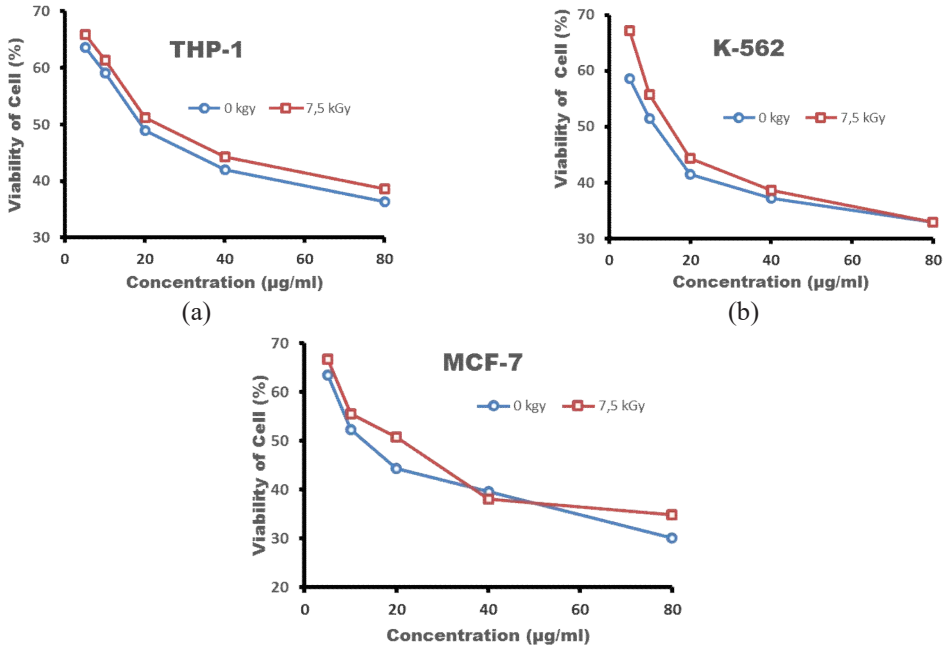

FIGURE 1. Relationship between the viability of (a) THP-1, (b) K-562, and (c) MCF-7 cell line, with the variation concentration of *G.ulmifolia* between 0 kGy (control) and irradiated 7.5 kGy.

The determination of cytotoxic activity was done and the IC_{50} value must be determined. IC_{50} is the result of the linear regression between the concentration with a probit table, the probit value can be obtained from the inhibition of the number living cell (Figure 2). IC_{50} is an inhibitory value of a substance by 50% of the tested cell or animal. Herbal plants have anti-cancer properties if they have an IC_{50} value <30 µg/ml which refers to the standard of the National Cancer Institute (NCI) (12). The results of the cytotoxicity activity of *G.ulmifolia* as a potential anti-cancer drug, the irradiation dose used was 7.5 kGy in *G. ulmifolia* still has toxic properties to cancer cells with IC_{50} below <30 µg / ml (Table 2).

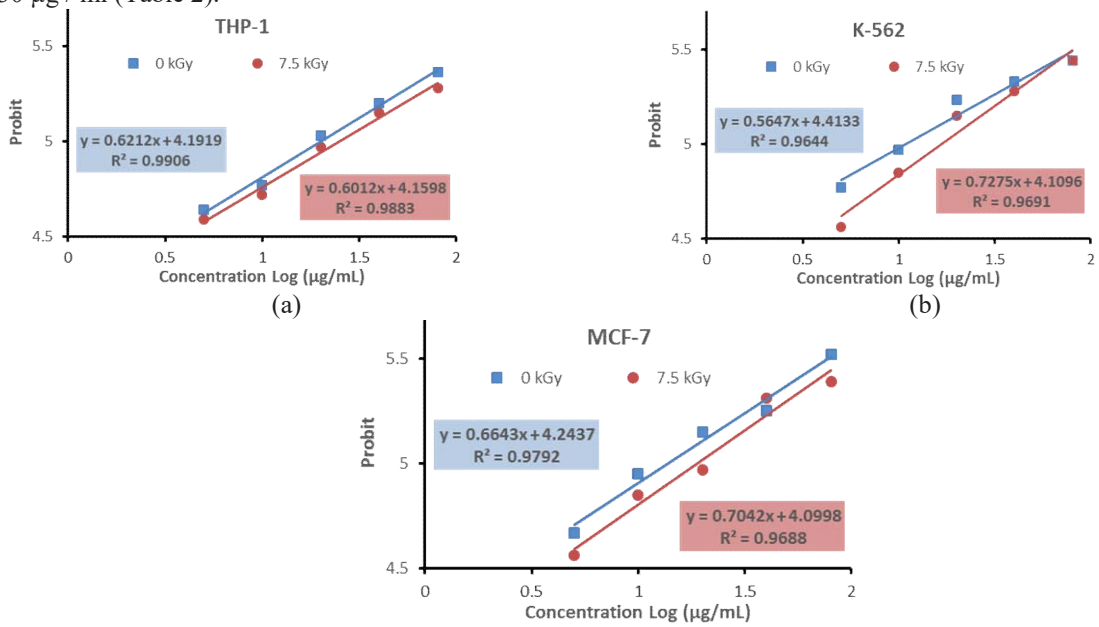

FIGURE 2. The linear regression and R from (a) THP-1, (b) K-562, and (c) MCF-7 cell lines, with the variation concentration of *G.ulmifolia* between 0 kGy (control) and irradiated 7.5 kGy.

Cytotoxic activity of *G.ulimofolia* ethanol extract on the cell line gave IC_{50} values differently (Table 2). The smallest IC_{50} is active in inhibiting K-562 cancer cells, the increase in IC_{50} is proportional to the increase in radiation dose received by *G. ulmifolia*. The decrease in IC_{50} is due to a decrease in the number of antioxidants or secondary metabolites in the plant. Secondary metabolites are very sensitive to gamma irradiation, at doses of 5 to 10 kGy the secondary metabolites will change and will become stable at a radiation dose of 20-30 kGy (13).

TABLE 2. Increase of IC_{50} value and decrease of antiproliferative activity against a cancer cell line

Cancer Cell line	IC_{50} (µg/ml)		Increase of IC_{50} value (%)	Decrease of antiproliferative activity (%)
	Control	7.5 kGy		
THP-1	19.99	24.97	30.5	23.4
K-562	10.94	16.74	53.0	34.6
MCF-7	13.75	18.97	37.9	27.5

Antioxidant compounds in *G. ulmifolia* around 88.52% which have an important role in damaging cancer cells by apoptosis. Apoptosis in cancer cells can be seen with cancer cell shrinkage, cancer cell shrinkage is caused due to damage to the mitochondria of cells that result in the death of cancer cells (14). The main content of *G.ulmifolia* is a flavonoid compound of 2.35%, this compound plays a major role in inhibiting the growth of cancer cells so that it can act as an immunomodulator which will kill cancer cells directly without affecting normal cells (15).

Gamma irradiation on herbal plants (*G.ulmifolia*) will affect the content of secondary metabolites in these plants. The use of irradiation doses up to 20 kGy is still relatively safe because the decrease in the content of secondary metabolites (flavonoid) does not have a significant difference compared to those without irradiation (control) so that it still has the same properties (1).

CONCLUSION

Gamma irradiation on *G. ulmifolia* with irradiation dose up to 7.5 kGy still has the same anti-cancer properties as control (without irradiation), so it can be concluded that irradiation dose of 7.5 kGy can be used as a preservation method for *G. ulmifolia* .

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