

## RESEARCH ARTICLE

# Effect of White Turmeric Extract (*Curcuma zedoaria*) Using Zam-zam Solvent Compare with Ethanol Solvent Against Breast Cancer Cell T47D

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### ABSTRAK

**Pendahuluan:** *Curcuma zedoaria* (kunir putih) adalah tanaman herbal berpotensi dalam melindungi dan menyembuhkan banyak penyakit khususnya sebagai antitumor dan antikanker. Kandungan senyawa dalam tanaman yang dipercaya dapat berpotensi terhadap kematian sel kanker adalah flavonoid, fenolik dan saponin. **Tujuan:** untuk mengetahui efek sitotoksitas ekstrak *Curcuma zedoaria*. **Metode:** Penelitian ini menggunakan jenis penelitian *Quasi eksperimental* dengan rancangan *post test non equivalent control group design* pada *cell-line T47D* (kultur sel kanker payudara) di laboratorium Biologi, Fakultas Kedokteran, Universitas Islam Sultan Agung, Semarang yang dibagi menjadi 2 kelompok. Kelompok 1 yaitu diberikan ekstrak *Curcuma zedoaria* pelarut zam-zam dan kelompok 2 diberikan ekstrak *Curcuma zedoaria* pelarut etanol dengan 10 variasi dosis yaitu 1,000 µg/mL; 500 µg/mL; 250 µg/mL; 125 µg/mL; 62,5 µg/mL; 31,25 µg/mL; 15,62 µg/mL; 7,81 µg/mL; 3,90 µg/mL; 1,95 µg/mL. Uji sitotoksik untuk memperoleh dosis IC<sub>50</sub> diperiksa dengan metode *direct counting* yang diolah dengan analisis probit.

**Hasil:** Hasil penelitian menunjukkan nilai IC<sub>50</sub> ekstrak *Curcuma zedoaria* pelarut zam-zam sebanyak 28,24 µg/ml sedangkan nilai IC<sub>50</sub> ekstrak *Curcuma Zedoaria* pelarut etanol sebanyak 13,71 µg/ml pada waktu inkubasi selama 24 jam. Hasil uji *Chi-Square* sebesar 0,000 atau nilai  $p < 0,05$  artinya terdapat hubungan secara bermakna.

**Kesimpulan:** Aktivitas ekstrak *Curcuma zedoaria* pelarut etanol tergolong sangat aktif sedangkan ekstrak *Curcuma zedoaria* pelarut zam-zam tergolong cukup aktif dan keduanya memiliki efek sitotoksitas pada kanker payudara sehingga proses apoptosis kemungkinan dapat terjadi.

**Keywords:** T47D, sitotoksitas, curcuma, zedoaria, zam-zam

### ABSTRACT

**Introduction:** *Curcuma zedoaria* is one of the herbal plants potentially protects and cures many diseases, particularly as anti-cancer and anti-tumor. Anti-cancer active compounds of it are flavonoid, phenolic, and saponin. **Objectives:** This study aimed to explore the cytotoxicity of *Curcuma zedoaria* extract (CZE).

**Methods:** *Experimental Quasi with post test non equivalent control group design* on T47D cell line at Biology laboratory, Faculty of Medicine, Sultan Agung Islamic University, Semarang. The research was consisted of 2 groups, namely: intervention group with CZE zam-zam solvent and intervention group with CZE ethanol solvent given 10 different dosages each for 1.000 µg/mL; 500 µg/mL; 250 µg/mL; 125 µg/mL; 62.5 µg/mL; 31.25 µg/mL; 15.62 µg/mL; 7.81 µg/mL; 3.90 µg/mL; 1.95 µg/mL. Cytotoxicity test of IC<sub>50</sub> using direct counting method and analyzed by probit analysis.

**Results:** IC<sub>50</sub> value of CZE in zam-zam and ethanol solvent were 28.24 µg/ml and 13.71 µg/ml respectively at the same 24 hours incubation period. Chi-square test revealed  $p$  value was 0.000 ( $\alpha = 0.05$ ), meaning that there was significant relationship.

**Conclusion:** CZE activity using ethanol is highly active while CZE using zam-zam water is moderate and both of them have the toxicity on breast cancer cell. As the result, apoptosis process may occur.

**Keywords:** T47D, cytotoxicity, curcuma, zedoaria, zam-zam

### INTRODUCTION

High cost and many side effects are the current problems on cancer treatment using chemotherapy, radiotherapy, and surgery (Baratawijaya, 2012). The side effects of chemotherapy includes a decrease in the number of erythrocytes, leucocytes, and thrombocytes;

loss of hair and eyelashes, mucositis (gastrointestinal/oral injury), and disorders of the peripheral nerves such as numbness, and tingling in fingers and toes (Dharmais, 2009).

Cancer has becomes world's problems. In 2008, the death rate from cancer was 17 million (Peter, 2008).

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*Pathological Based Registration* (2010) in Indonesia stated that breast cancer ranks number 2 after cervical cancer with a relative frequency of 11.5%, which means 11-12 new cases per 100,000 population were at risk (Manuaba, 2010).

Phytochemical content of *Curcuma zedoaria* includes phenolic, saponin, flavonoid, and terpenoid. The phenolic antioxidants are stable and commonly used in cancer prevention. Phenol affect apoptosis by inhibiting the expression of regulatory proteins, inhibiting DNA damage, the cells proliferation, and inhibit the Her-2 gene/*neu* which caused breast cancer. Based on research, flavonoid compounds in herbal medicine has the effect of blocking the growth receptor, and inhibited the MAPK (Mitogen-Activated Protein Kinase), in Tyrosine Kinase Receptor (TKRs) signaling pathway (Hiroko, 2002).

This study was conducted to test the cytotoxicity of white turmeric (*Curcuma zedoaria*) extract (CZE) in T47D cell culture, which is an in vitro model for breast cancer cells. The growth inhibitory activity of T47D breast cancer cell line using *Curcuma zedoaria* extract in Zam-Zam solvent compared with ethanol at various dose.

## METHODS

This research used quasi experimental study with post test non-equivalent control group design on the T47D cell-line (breast cancer cell culture) in the laboratory of Biology, Faculty of Medicine, Sultan Agung Islamic University, Semarang, which were divided into 2 groups. Group 1 was given *Curcuma zedoaria* extract (CZE) in zam-zam solvent. While the second group was given CZE in ethanol solvent. Each group were given 10 different doses of 1,000 µg/mL; 500 µg/mL; 250 µg/mL; 125 µg/mL; 62.5 µg/mL; 31.25 µg/mL; 15.62 µg/mL; 7.81 µg/mL; 3.90 µg/mL; 1.95 µg/mL.

### Steps:

Cytotoxicity test by "direct counting" method performed in the Biology laboratory. The extract was made at the Chemistry laboratory of Faculty of Medicine, Sultan Agung Islamic University, Semarang. Materials used were 10-12 months of white turmeric rhizomes's (*Curcuma zedoaria*) powder weighing 50 grams, zam-zam as solvent, ethanol 99%, infundation instruments, temperature gauge, Wharton Paper No. 1, a water bath, and an analytical scale.

Fifty grams white turmeric (*Curcuma zedoaria*) powder were infundated with 300 ml of zam-zam solvent for 15 minutes, then filtered using a paper



Figure 1. CZE in zam-zam and ethanol solvent were diluted with RPMI 1460 medium at T47D cell line cytotoxicity test.

Wharton 1. The infuse result were then underwent evaporation for 15 minutes at 900C resulting a thick extract weigh 2.026 grams.

On the other hand, 50 grams powder of white turmeric (*Curcuma zedoaria*) underwent maceration using 99% ethanol for 24 hours, and then filtered using a paper Wharton 1. A hundred and fifty milliliters of the extract were then processed by Rotary evaporator (Heidolph-Japan) at a water bath in temperature 500C, coolant temperature set 200C, as well as the frequency of round gourd rotary 95 rpm for 30 minutes to produce 0.729 grams of extract.

Dilution with RPMI1460 medium was done to test the cytotoxicity on the T47D cell line. Ten doses for cytotoxicity assay of CZE in zam-zam and ethanol solvent were 1.000 µg/mL; 500 µg/mL; 250 µg/mL; 125 µg/mL; 62.5 µg/mL; 31.25 µg/mL; 15.62 µg/mL; 7.81 µg/mL; 3.90 µg/mL; 1.95 µg/mL. Incubation was performed for 24 hours.

T47D Cell line were thawed, initiated, then after incubation for 4 x 24 hours in a 5% CO<sub>2</sub> incubator at 370C, cell is 80% already confluent and ready to be harvested and counted. Furthermore, the treatment with 10 various doses of the CZE was repeated three times. After 24 hours incubation, eosin staining on a hemocytometer was performed.

*Direct counting method was able* to determine which were the dead or the viable cells. Dead cells were red stained, while the viable cells are highlighted. Cells were counted under a light microscope with a 400x magnification in in 5 visual fields.

## Data Analysis

Cytotoxicity effects (IC<sub>50</sub> values) of CZE in zam-zam and ethanol solvent were obtained from the probit

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**Table 1.** The mean percentage of viable cells of T47D cell line after treatment with CZE in zam-zam and Ethanol solvent.

Dose (µg/mL)	The mean percentage of viable cells (%)	
	CZE in Zam Zam Solvent	CZE in ethanol solvents
1000	0	0
500	0.31	0
250	0.94	0
125	2.82	11.59
62.50	3.76	11.59
31.25	26.95	22.88
15.62	56.42	35.10
7.81	87.46	56.73
3.90	87.77	66.14
1.95	70.53	74.92

statistical analysis.  $IC_{50}$  value indicates the percentage of cell death in culture, and the result was 50% (Ma'at, 2011).

## RESULTS

Direct counting methods by haemocytometer eosin staining to determine the viable cells, which then used to determine  $IC_{50}$  dose of CZE). Probit analysis is then performed.

From table 1, it can be inferred that there has been a T47D cells death due to CZE. Based on the analysis of probit, cell death occurred in a 24-hour incubation with the value  $IC_{50}$  CZE in zam-zam solvent by 28.24 µg/ml. While based on the statistical analysis of probit of CZE in ethanol solvent,  $IC_{50}$  values were 13.71 pg/ml at 24 hours incubation.

## DISCUSSIONS

The results indicated that CZE in zam-zam solvent have  $IC_{50}$  values of 28.24 or 20 µg/ml  $<IC_{50} <100\mu\text{g/ml}$ , defined as fairly active category (Tanamatayarat, 2003). Meanwhile, the results also showed that CZE in ethanol solvent have  $IC_{50}$  values of 13.71 pg/ml or  $IC_{50} <20\ \mu\text{g/ml}$ , defined as very active category (Tanamatayarat, 2003). CZE in ethanol solvent were more potent than zam-zam solvent, both of them have are potential against cancer cell death.

It is alleged that flavonoids, phenolics and saponins contained in CZE resulting in toxic effects on the T47D cell line. Phenolic substances often used in cancer prevention, since it was able to affect apoptosis by inhibiting the expression of regulatory proteins, inhibiting DNA damage, cells proliferation, and the Her-2/*neu* gene that cause breast cancer. Phenolic compounds have been demonstrated to have an anti-proliferation effect of T47D cells via the extrinsic pathway system Fas/FasL, showed inhibition of cell growth, but this depends on the duration and dose given (Kampa, 2004).

## CONCLUSION

It is concluded that the CZE in Zamzam solvent is potential as an anticancer agent, with  $IC_{50}$  doses of 28,24 µg/ml, whereas CZE in ethanol solvent have  $IC_{50}$  doses of 13.71 pg/ml, both at 24 hours incubation time. CZE have a cytotoxic effect for their flavonoids and phenolic compounds, act as antioxidants.

## SUGGESTION

Further research are needed to compare efficacy against cancer cells between zam-zam solvent and water.

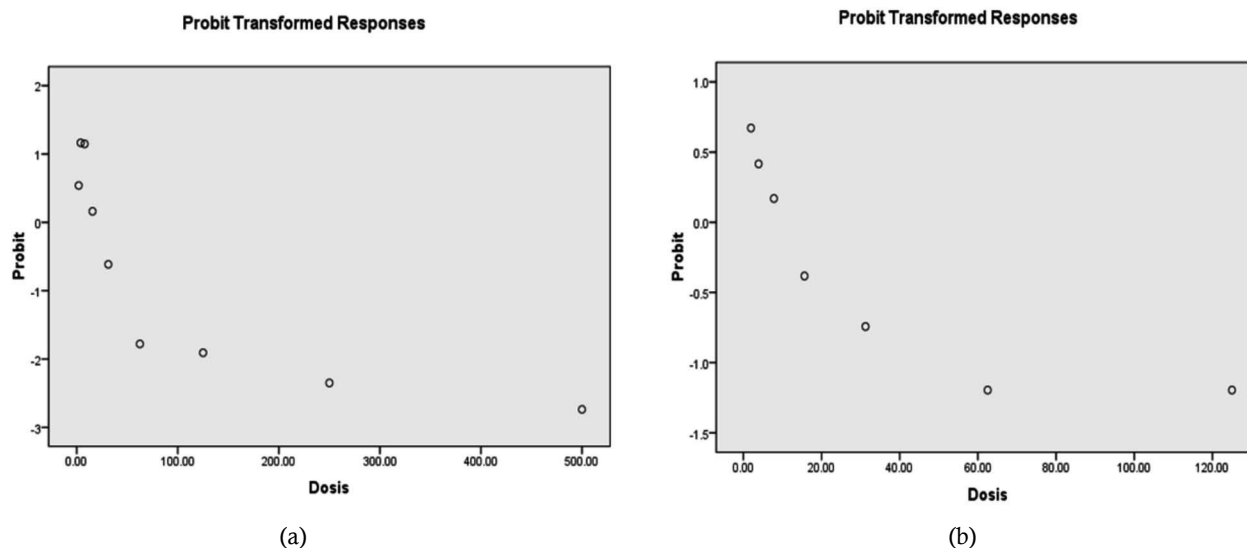


Figure 2. Cytotoxicity graph of CZE in zam-zam solvent (a), and in ethanol solvent (b) on T47D cell line with various doses.

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