# In Vitro Antiproliferation Activity of Temulawak *(Curcuma xanthorriza* Roxb.*)* Ethanol Extract on YAC-1 and HeLa Tumor Derived Cell Lines

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## Introduction

A tumors or neoplasm can be defined as a disturbance of growth characterized by excessive, abnormal and uncontrolled proliferation of transformed or altered tissue at one or more primary points within the host, and frequently at one or more metastatic sites. Natural metabolites especially from plants are widely use for medical purposes. In some Asian countries, the use of plants for traditional medicine in the treatment of some disorders in human and animal is a common practice. *C. xanthorriza* known as "Temulawak" in Indonesian language is one of 7,000 Indonesia's medicinal plants out of 30,000 plants found in Indonesia; this number accounted as a 90% of Asia's medicinal plants (1) Ethanol extracts of the root of *C. xanthorriza* (Figure 1) was known had an antiproliferation activity on canine tumor-derived cell lines (2). This plant extract gave a significant inhibition of cell growth activity on both canine tumor cell lines. The aim of this study is to elaborate the anti-proliferation effect of 70%-ethanol extracts from *C. xanthorriza* on tumor-derived cell lines in order to find the anti-tumor drugs for medical purposes both in human and animal.

Fig. 1: The plant with flower (left) and root (right) of *C. xanthorriza* (source: www.geocities.com)

## Materials and Methods

*Brine Shrimp Lethality Test:*Ten larvae of *Artemia salina* on 18 vials each were used (5 concentrations of extracts and one control with 3 replicates). After 24 hours of extracts treatment, the dead *A. salina* was counted. The data were processed statistically using Probit Test.

*Cell Culture:* Cell lines were cultivated in a 24-well culture plate on DMEM-F12 supplemented with 10% FCS and antibiotics (100 IU/mL penicillin, 100 g/mL streptomisin) with density of $10^5$ cell/mL. Cells were then exposed to 6 different concentration of the extract i.e. 0, 15, 30, 45, 60, 75 ppm; and doxorubicin was used as a control positive. Treated cells were plated in 3 replicates. Cells were then incubated at 37ºC with 5% $CO_2$ in air.

*Cell Harvesting & Counting :* Cells were harvested after 4 days in culture when confluence was achieved. Total cells from each treatment were counting using a hemacytometer with Trypan Blue dye exclusion and the cell numbers were averaged. The antiproliferation activity was then calculated.

## Result and Discussion

*Anti-proliferation Activity* : Exposured of YAC-1 and HeLa cells with gradual concentrations of *C. xanthorriza* extract resulted in the increasing of the cell growth inhibition (Figure 2), this condition indicated that there was an antiproliferation activity of this extract to these both cell lines.

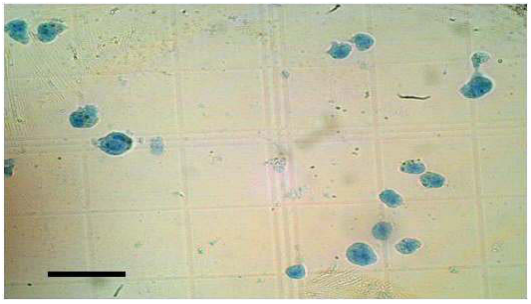

Fig. 2: Dead tumor cells on the hemacytometer counter. Trypan blue dye. (bar = 40 µm).

The degree of this activity was varied in each cell lines. The highest anti-proliferation activity of *C. xanthorriza* ethanol extract on each cell lines were 70.0% on YAC-1 cell line and 37.41% on HeLa cell line (Figure 3). This activity was occurred on the dose of 75 ppm.

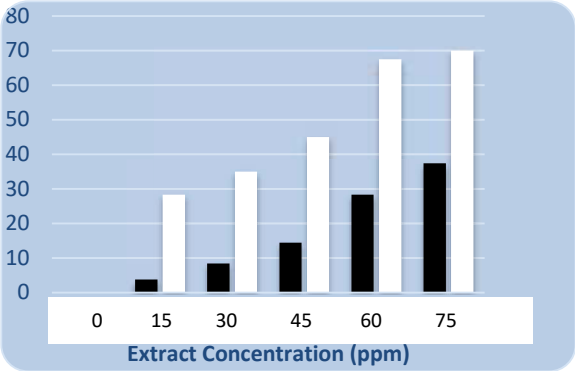


Fig. 3: Antiproliferation activity of *C. xanthorriza* extract on YAC-1 (white box) and HeLa (black box) cells.

*C. xanthorriza* had a main bioactive substaces of curcuminoid and others (3). Curcuminoid give the root a yellow colour and had an anti-bactery, anti-cancer, anti-inflamation, anti-oxidant and hypocholestemic (4). The other components in *C. xanthorriza* were consists of camphor, mirsen, xanthorizol, β-curcumin, arcurcurmin, isofuranogermakren and p-toluilmethylcarbinol (5). Xanthorrizol combined with curcumin were the main substances that acts as potential bioactive compound of *C. xanthorriza* (3). Ethanol extract of *C. xanthorriza* has an activity in inhibiting the growth of MCA-B1 and MCM-B2 derived canine-tumor cell lines with inhibition ranging from 70% – 75% at the extract concentration of 75 ppm (2). Curcumin was reported could inhibit the cell proliferation of several tumor cells i.e . HL-60, human leukemia, depend on the dose and time exposure (6). Curcumin inhibited cell proliferation by stimulating the apoptotic mechanism through mitochondrial pathway involving activation of Caspase-8, BID cleavage, Cytochrome C release dan Caspase-3. Curcumin also inhibited the induction of nitrate oxide synthesis within the activated-macrophages. Curcumin showed their activity on anti-cancer by reducing the number of nitrate oxide or iNOS (inducible nitric oxide synthase) which known as one of the initiator of the tumor formation. NF-kappa-B is involve in the induction of iNOS, caused oxidative stress, which know as one of the tumor initiator. Curcumin acts by inhibit the phosphorylation and degradation of kappa-B-alpha inhibitor through a mechanism by inhibiting the activation of NF-kappaB, where the result will decreasing the transcription of iNOS gene (7). Curcumin could inhibit *lipooxygenase* (LOX), *cyclooxygenase* (COX)-1 dan COX-2 as well as lipopolysacharides that will terigered the COX-2 expression (6). In tumor cells, the excesive expression of COX-2 which resulted in the over production of prostanoid will caused increasing the proliferation and inhibit the process of apoptosis (8). Increasing in cell proliferation is occured due to activation of several oncogene that involved in the mitogenic signal such as Ras oncogene. Inhibition on the apoptotic process is due to the effect of the excessive expression of Bcl-2 oncogene. Inhibition of COX caused the prevention of excessive prostanoid production by curcumin and resulted in the decreasing of inflammation effect, preventing tumor cell proliferation and enhance the apoptosis process.

In this pathway, apoptotic process is stimulated by the accumulation of acid arachidonat. Accumulation of this acid will activate *sphingomyelinase* enzyme which catalyze the production of ceramid from *sphingomyelin* and finally ceramid will stimulate the apoptotic process. Curcumin also capable in the inhibit the initiation process of the tumor formation due to benzo(a)pirene (8). This chemoprevention effect is due to curcumin has an ability in the inhibit the activity of cytochrome P450 and gluthation-S-transferase which causing the inhibition of activation of benzo(a)pirene as a mutagenic substances.

Xanthorrizol, a sesquiterpen component in *C. xanthorriza* could increasing apoptotic process on HeLa cells by assayed using a TUNEL method as well as nuclear morphology using a Hoechst 33258 stain (9). Xanthorrizol did not influenced the expression of anti-apoptosis protein (Bcl-2) and viral oncoprotein E6. Xanthorrizol is a substance that functioned as an anti-proliferative and anti-cancer through a mechanism by apoptotic induction of p53 and Bax on the HeLa cells. Based on all findings mentioned above, we concluded that *C. xanthorriza* root-ethanol extract has an antiproliferation activity on YAC-1 and HeLa tumor-derived cell lines, and this phenomenon could be further studied for the widely used of this plant extract in the combating of tumor disorders both in human and animals.



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