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The Anti-proliferation Assay of Bioactive Fraction from *Curcuma zedoaria* Rhizome

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Abstract

This study was aimed to investigate the anti-proliferation activity of the bioactive fraction from Curcuma zedoaria. The dried powder of *Curcuma zedoaria* rhizome was extracted using continuous maceration method with n-hexane, ethyl acetate and methanol 50%, respectively. Brine Shrimp Lethality Test (BSLT) has been used as a preliminary test for screening the antiproliferation activity of three extracts. Ethyl acetate extract showed the highest activity of all and it became the subject for fractionation using column chromatography with silica gel GF as stationary phase and the combination of n-hexane:ethyl acetate in various gradient concentration as mobile phase. The fractionation process produced five fractions, namely fraction A, B, C, D and E. The anti-proliferation assay of the resulted fractions was determined with tumor cell lines of HeLa, K562 and WEHI 164. The study found that fraction C (15 ppm) could inhibit the proliferation of HeLa cells by 32%, K562 cells by 55% and WEHI cells by 49%.

Key words: *Curcuma zedoaria*, bioactive fraction, anti-proliferation

Introduction

It has been found recently that *Curcuma zedoaria* (Zingiberaceae) or known as temu putih, partcularly the rhizome part, has been used traditionally for cervical cancer treatment and increasing chemotherapy effectiveness (Dalimartha 2003). The rhizome of temu putih contains chemical constituents including essential oil, curcuminoid (diarylheptanoid), polysaccharide and other active substances (Tang dan Eisenbrand 1992). As a part of traditional medicines, the rhizome of temu putih has been used as stimulant, carminative, diuretic agent, antiemetic, antipiretic, antidiarrhea, gastrointestinal disorders reliever, healing agent for ulcer, wound and other skin diseases. (de Padua *et al*.1999). Occasionally, the use of temu putih rhizome for treating tumor has been combined with other remedies like temu mangga (kunir putih), temu lawak, benalu teh, delimaputih, pulosari, sambung nyawa, beluntas and others in accordance to the purpose of the treatment (Syukur 2002, Suharmiati *et al.* 2002).

Some investigational research reported that the formulation of temu putih rhizome can inhibit the growth of myeloma cells, carcinoma cells (Priosoeryanto *et al.* 2001), inhibited lung tumor in benzo(a)piren-induced mice (Murwanti *et a.l* 2004). The research on bioactive component of temu putih included the analysis of essential oil (Sudibyo 2000), antibacterial activity of essential oil (Sunardi et al 2002, Wilson 2005), analgesic and anti-inflammatory activities of curcumenol (Navarro *et al.* 2005, Jang *et al*. 2004), anti-proliferation activity of curcuminoid to OVCAR-3 cells (Syu *et al* 1998).

This research was aimed to elucidate the potency of biological activity from bioactive component of temu putih rhizome as anti-proliferative agent to epithelial cancer cell, blood cell cancer (leukemia) and connective tissue (fibroblast) cancer.

Methodology

The Extraction and Fractionation

The dried powder of *Curcuma zedoaria* rhizome (1 kg) was extracted using continuous maceration method with n-hexane, ethyl acetate (EtOAC) and methanol (MeOH) 50%, respectively. Brine Shrimp Lethality Test (BSLT) has been used as a preliminary test for screening the antiproliferation activity of three extracts.

Ethyl acetate extract showed the highest activity of all and it became the subject for fractination using colom chromatography (75x4,5 cm) with silica gel GF as stationary phase and the combination of n-hexane:ethyl acetate in various gradient concentration as mobile phase. The fractionation process produced five fractions, namely fraction A, B, C, D and E.

Brine Shrimp Lethality Test.

The assay was performed as described previously (Meyer *et al*., 1982) using brine shrimp (*Artemia salina* Leach) nauplii. The eggs were placed in brine and hatched within 48 h. Each extracts and fractions (2 mg) were dissolved in 20 mL EtOAC to prepare a stock solution of 0.1 ppm concentration. From the stock solution, 200, 20, 2, and 0,2 mL were transferred in triplicate to vials and the solvents were allowed to evaporate. After evaporation, 5 mL of brine was added to each vial (10 shrimps per concentration). The number of survivors at each concentration was recorded and the LC_{50} values were determined from the plotted graph of mortality percentage against the log concentracion of each sample.

Anti-proliferation Activity assay

In vitro antiproliferation was measured with HeLa (human epithelial cells), K-562 (CML meiloid), WEHI 164 (fibroblast cell sarcoma). The cell lines were cultured with the density of 10^3 cell/mL on the 24-well dish using a growth medium compromised from DMEM and 10% FCS (Priosoeryanto *et al.* 1995.). The tested dose of each fractions was determined after the LC_{50} of each fractions were recognized. The dose for each fractions are as follows: A and D fraction: 30 ppm; B and C fraction: 15 ppm; E fraction; 75 ppm. The fractions were added to the cultured dish 3 holes for each dose. After the conluence of cell growth was achieved on the control negative dishes, the cells were harvested and the average of the total number of cells on each dishes were counted using a hemacytometer with Tryphan Blue dye. The data were then statistically analyzed to determine the anti-proliferation level.

Results and Discussions

The Extraction and Fractionation

The extraction of the powder from *C. zedoaria* rhizome produced rendemen (the ratio of resulted extract to initial powder of temu putih rhizome) of 10.4% (n-hexane extract), 7.2% (ethyl acetate extract) and 5.5% (methanol extract. The rendemen of hexane extract was the highest of all. This result conformed to the main component of zingiberaceae family , namely essential oil where it could be isolated from *C. Longa* and *C.xanthorrhiza* with essential oil concentration of 3-4% and 8-10% respectively (Liang 1992), whereas in *C. zedoaria* around 1-2,5% of essential oil could be isolated (Dalimartha 2003).

The chemical constituents of essential oil from C. *zedoaria* were monoterpen (α-pinen, D-kamfen, D-borneol, D-kamfor, sineol) and seskuiterpen (bisabolan, eleman, germakran, eudesman, guaian, spirolakton (Windono dan Parfati 2002). The result phytochemical screening revealed that the powder from temu putih rhizome contained the chemical substances groups, namely essential oil, steroid, terpenoid, flavonoid, tannin and saponin. Of three extract tested, the chemical substances could be found in each extract in accordance to the polarity degree of the extract, for example n-hexane extract contained essential oils, steroid, terpenoid, in ethyl acetate contained steroid, terpenoid and flavonoid while tannin and saponin could be isolated from methanol extract.

The fractionation of ethyl acetate extract (60 gram) yielded 5 fractions included fraction A (9.30 gram), fraction B (0.87), fraction C (0.99 gram), fraction D (13.77 gram) and fraction E (33.90 gram). Fraction A contained non-polar compound in association with

the polarity gradient of mobile phase system (n-hexane:ethyl acetate) initiated with n-hexane, whereas a great proportion of semi polar compound could be found in fraction E.

Brine Shrimp Lethality Test.

The LC_{50} for each extract *Curcuma zedoaria* were 155 ppm (hexane extract), 100 ppm (EtOAc extract) and 248 ppm (MeOH extract). An EtOAc-extract *C. zedoaria* showed significant activity against brine shrimp with LC_{50} value of 100 ppm (Figure 1).

McLaughlin and Rogers (1998) reported that the positif relationship between Brine Shrimp Lethality Test with cytotoxicity assay 9KB cell (*human nasopharyngeal carcinoma*) and the test was used as prescreen in *Laboratory of the Purdue Cancer Center.*

The LC_{50} for each fraction extract EtOAc (Figure 2) were 27 ppm (A fraction), 17 ppm (B fraction), 14 ppm (C fraction), 31 ppm (D fraction) and 73 ppm (E fraction).

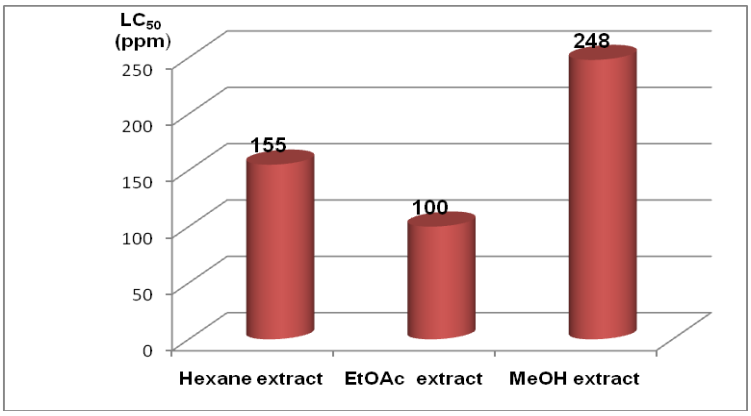

Figure 1. LC_{50} value of some extract of *C. zedoaria*

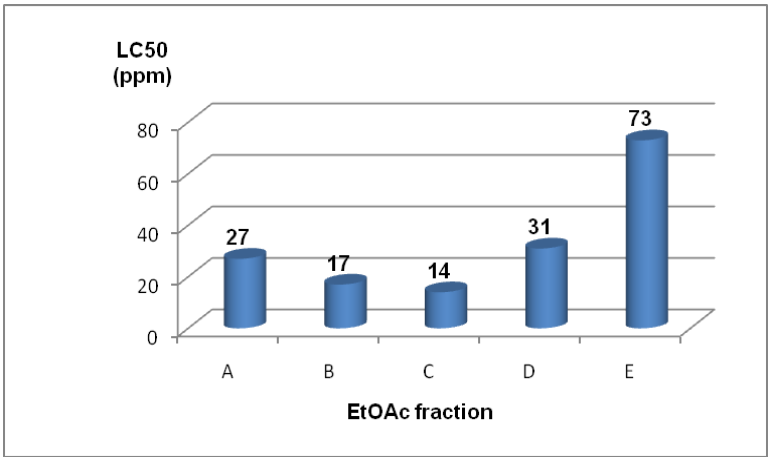

Figure 2. LC_{50} value of fraction of EtOAc extract from *C. zedoaria*

Anti-proliferation Activity

The anti-proliferation activity was detected in all fraction EtOAc extract *C. zedoaria*. The degree of this activity on all fraction was varied. The highest anti-proliferation activity of

fraction EtOAc on each cell lines were 32% for HeLa cell, 55% for K-562 cell and 49% for WEHI-164 cell, this activity was occured on the dose of 15 ppm C fraction (Figure 3).

The anti-proliferation activity pattern of B dan C fraction on HeLa, K-562 and WEHI-164 cell lines was generally similar with the same doses. The similar anti-proliferation activity on tumor cells as B and C fraction was also encountered in the A and D fraction with the same dose at 30 ppm. Hutapea (1993) explain that *C. zedoaria* contain volatile oils, saponin, flavonoids and polyfenol. Syu et all (1998) reported that curcuminoid from ethanol extract C. zedoaria have anti-proliferation activity against OVCAR-3 cell. Accoring to Suffness and Pezzuto (1991) fractions of B and C were classifief as active fractions with Anti-Proliferation Activity (AP) tumor cell value around 50 % in concentration < 20 ppm.

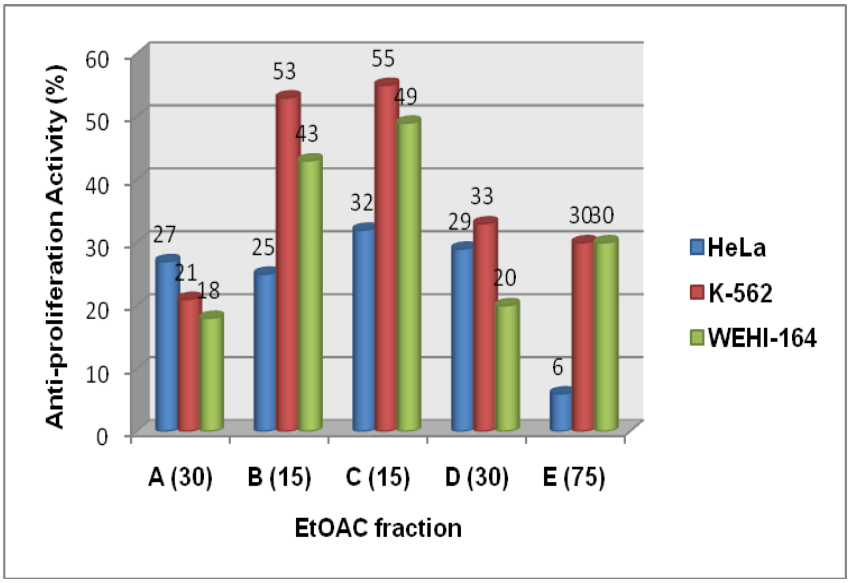

Figure 3. Anti-proliferation activity of EtOAc fraction on HeLa, K-562 and WEHI-164 cell lines.

Conclusion

The result of this study showed that the B and C fraction *C. zedoaria* have an anti-proliferation activity on some tumour –derived cell lines. The highest anti-proliferation activity of C fraction EtOAc (dose 15 ppm) on each cell lines were 32% for HeLa cell, 55% for K-562 cell and 49% for WEHI-164 cell. We suggest the futher study on isolation and identification of the bioactive compound.

References

Dalimartha S. 2003. *Atlas tumbuhan obat Indonesia*. Jilid 3. Jakarta: Puspa Swara.

De Padua LS, Bunyapraphatsara N and Lemmens RHMJ (editors).1999. Plant resources of South-East Asia, Medicinal and poisonous plants. *Prosea Publ* 12(1):216-219.

Hutapea,JR. 1991. Indonesian medicinal plants. Vol I

Jang MK, Lee JH, Kim JS, Ryu J-H. 2004. A curcuminoid and two sesquiterpenoids from *Curcuma zedoaria* as inhibitors of nitric oxide synthesis in activated macrophages. *Arch Pharm Res* 27(12):1220-1225.

Liang OB, penemu; PT Darya-Varia Laboratoria Jakarta.9 Juni 1992. Combinations of compounds isolated from Curcuma spp as anti-inflmmatory agents. ID 5 120 538.

McLaughlin JL, Rogers LL. 1998. The use of biologycal assays to evaluate botanicals. *Drug Inform J* 32:513-524.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaugghlin JL. 1982. Brine shrimp: a convinient general bioassay for active plant constituens. *Hippokrates* (45)31-34.

Murwanti R, Meiyanto E, Nurrochmad A, Kristina SA. 2004. Efek ekstrak etanol rimpang temu putih (*Curcuma zedoaria* Rosc.) terhadap pertumbuhan tumor paru fase inisiasi pada mencit betina diinduksi benzo(a)piren. *Majalah Farmasi Indonesia* 15(1): 7-12.

Navarro DF, Souza MM, Neto RA, Golin V, Niero R, Yunes RA. 2002. Phytochemical analysis and analgetic properties of Curcuma zedoaria grown in Brazil. *Phytomedicine* 9:427-432.

Priosoeryanto BP, S Tateyama, R.Yamaguchi and K. Uchida.1995. Antiproliferation and colony-forming inhibition activities of recombinant feline interferon (rFeIFN) on various cells in vitro.*Can J Vet Res* 59:67-69

Priosoeryanto BP, Sumarny R, Rahmadini Y, Nainggolan GRM, Miswidia, Andani S. 2001. *Growth inhibition effect of plants extract (Mussaendah pubescens and Curcuma zedoaria) on tumor cell lines in vitro.* Di dalam: *International Symposium-cum Workshop*.Phillippines, August 27-31, 2001.

Sudibyo RS. 2000. Gas chromatographic-mass spectrometric analysys of the main component of volatile oil isolated from *Curcuma zedoaria* Rosc. *Majalah Farmasi Indonesia* 11(1): 39-44.

Suharmiati, Handayani L, Roosihermiatie B. 2002. *Pengobatan kanker dan tumor dengan menggunakan ramuan tradisional yang mengandung kunir putih (Curcuma mangga Val. Et Zyp) (Studi kasus di Laboratorium Penelitian dan Pengembangan Pelayanan Obat Tradisional tahun 2000 sampai dengan 2001*. Di dalam: *Prosiding Seminar Nasional Tumbuhan Obat Indonesia XXI.* Surabaya:27-28 Maret 2002.

Suffness M, Pezzuto JM. 1991. *Assays related to cancer drug discovery*. Di dalam: Hostettmann K, editor. *Assays for bioactivity*. Academic Pr.

Sunardi C, Dewi PNL, Sutedja L, Kardono LBS. 2002. *Studi aktivitas antimikroba minyak atsiri dari rimpang Kaemperia rotunda L., Curcuma zedoaria Rosc. Dan Curcuma mangga Val.Et ZIJP.*Di dalam: *Prosiding Seminar Nasional Tumbuhan Obat Indonesia XXI.* Surabaya: 27-28 Maret 2002

Syukur C. 2002. *Temu putih tanaman obat antikanker*. Penebar Swadaya Syu WJ, Shen CC, Don MJ, OU JC, Lee GH and Sun CM. 1998. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*, *J Nat Prod* 61(12):1531-1534.

Tang W and Eisenbrand G.1992.*Chinese drugs of plant origin*. Springer Publishing.

Windono T, Parfati N. 2002. *Curcuma zedoaria* (Bergius) Roscoe: kajian pustaka dan aktivitas farmakologik. *Artocarpus* 2(1):1-10.