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Curcumin Analysis and Cytotoxic Activities of Some *Curcuma xanthorrhiza* Roxb. Accessions

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Abstract : Curcumin in rhizome of *Curcuma xanthorrhiza* Roxb. have potential pharmacological activities. In the present study, curcumin contents and cytotoxic activities of some *C. xanthorrhiza* accessions were evaluated. The curcumin contents were analyzed by HPLC. Cytotoxic studies using brine shrimp lethality test and Vero and MCF-7 cell line cultures were carried out. The curcumin content varied between 24.70 ± 10.72 mg g^{-1} in accession of SG (Sragen) to 54.09 ± 3.48 mg g^{-1} in accession of WG (Wonogiri). All accessions were found to be effective in general toxicity against brine shrimps. Accession of WG showed in vitro cytotoxicity against Vero and MCF-7 cell line. Accession of WG indicated the possibility of selecting high quality clone for curcumin production and anticancer in MCF-7 cell line.
Key words: Curcumin, MCF-7 cell line, BSLT, Vero cell line, *Curcuma xanthorrhiza* RoxB.,Temulawak.

Introduction

Curcuma xanthorrhiza Roxb., a well-known medicinal plant belonging to Zingiberaceae family, is used as a traditional herb in Indonesia[1]. Curcumin is active constituents contained in rhizome of *C. xanthorrhiza*[2]. In the literature, curcumin has been reported for pharmacological activities such as antioxidant[3], anti-inflammatory[3], anticancer[4], anti-diabetic[5], antibacterial[6], antiparasitic[7], and anti-depressant[8]. Evaluation of curcumin contents and bioactivities of the different *C. xanthorrhiza* accession remains unexplored and limited. Therefore, the current study aimed to evaluate variation of curcumin contents and cytotoxic activities of four *C. xanthorrhiza* accessions to determine the possibility with the best performance to be used as initial material in breeding program of plant improvement for use pharmaceutical industrial.

Experimental

The experiment was conducted from October 2013 to June 2014 at the Cihanyawar, Nagrak, Sukabumi, West Java, Indonesia (6°49'55.49"S, 106°49'3.09"E; average altitude of 1697 m). In this experiment, four accessions of *C. xanthorrhiza*, namely, WG (Wonogiri), KA (Karanganyar), SB (Sukabumi), and SG (Sragen), were used, together with one variety, used as control: Cursina III from BalaiPenelitianTanamanRempahdan Obat (Balittro, Bogor, Indonesia). The experiments were arranged in a completely randomized design with three replications. All rhizome of accessions and Cursina III of *C. xanthorrhiza* were grown at the same condition with 50 cm x 60 cm spacing and fertilized with 20 t manure ha^{-1}. Rhizome of each accession were harvested at nine month after planting. The rhizomes were washed in water, cut into small species and dried for ± 5 days

(moisture < 10%), and then ground into powder (size, 100 mesh). Dry-powderrhizomes (25 g) were extracted with 250 mL of ethanol 96%for 2x24 h. The residue of extraction was sequentially extracted by soxhlet with ethanol 96%. The ethanol extract was extracted by liquid-liquid extraction using n-hexane (1:1, v/v). Finally, ethanol fraction was concentrated by evaporation (BUCHI, R-250, Switzerland) at ±50 ºC and stored in tightly closed dark vials at 4ºC until analysis.

The curcumin content from ethanol fraction of *C. xanthorrhiza* accessions were analyzed by High Performance Liquid Chromatography (HPLC, HITACHI) based on method developed by Jayaprashka[9].

Preliminary screening of cytotoxic activity from ethanol fraction of *C. xanthorrhiza* accessions were used brine shrimp lethality test (BSLT) with concentration of 10, 100, 500, and 1000 µg mL^{-1} according the procedure described by Meyer[10].Cytotoxic activity was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma)assay[11], some modification, with using MCF-7 cancer cell lines (ATCC HTB 22) and non-cancerous Vero cell line (ATCC CCL 81) were obtained from Primate Research Center, Bogor Agricultural University.Cell lines were cultured in Dulbecco's minimum eagle medium (Gibco)supplemented with 10% fetal bovine serum (Sigma), 100 µg mL^{-1} penicillin (Ginco) and 100 µg mL^{-1}streptomycin (Ginco). Briefly, 2 x 10^3 cells mL^{-1}were exposed to different samples concentration (10 – 1000 µg mL^{-1}) in Vero cell and one sample concentration (13.75 µg mL^{-1}, 1/8 of IC_{50} Vero cells from sample with low cytotoxic) in MCF-7 cell for 72 h. The control group (untreated cells) was also included. Then, the medium was removed and added 20 µL MTT (2 mg mL^{-1}). After 4 h incubation, the reaction was added 100 µL HCl-isopropanol (0.1 N). The absorbance at 595 nm was measured with a microplate reader (Bio-Rad).

Analyses were performed in triplicates and the data expressed as means and standard deviation. Statistical analysis was performed by Statistical Tool for Agricultural Research, and the difference among *C. xanthorrhiza* accessions was analyzed with least significant difference (LSD) test. A significant difference was considered for $p < 0.05$.Similarity analysis among the accession were analyzed using hierarchical cluster.

Results and Discussion

Fig. 1 showed curcumin contents of ethanol fraction in Cursina III variety and some accessions of *C. xanthorrhiza*. Variation was observed in curcumin content of some accessions. The content of curcumin varied between 24.70 ± 10.72 mg g^{-1} in accession ofSG (Sragen) to 54.09 ± 3.48 mg g^{-1} in accession of WG (Wonogiri). No significant difference was observed in curcumin contents between Cursina III variety and accession of WG, KA and SB of *C. xanthorrhiza*, depicted with the same superscript in Fig. 1. Based on the result, WG accession is the clone with high curcumin production soits possibility to be used as initial material in plant breeding program for Indonesia pharmaceutical industry.

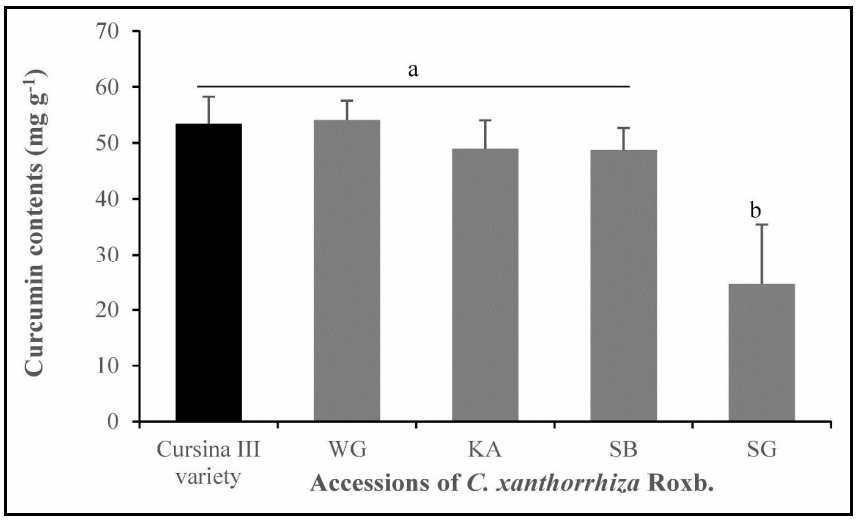

Fig.1. Curcumin contents (±SD) in Cursina III variety and some accessions of *C. xanthorrhiza*. Values followed by the same superscripts are not significantly different (p<0.05) by LSD test.

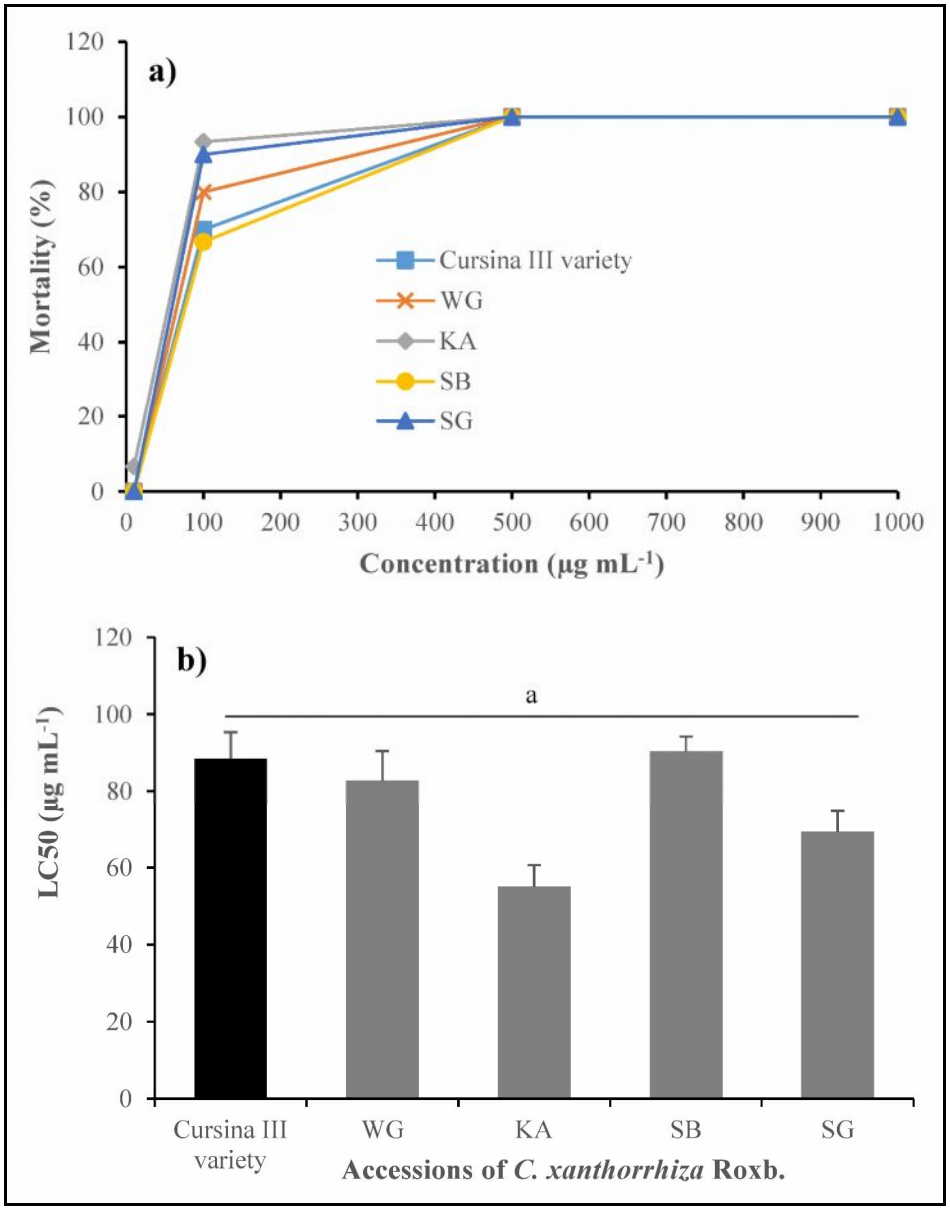

Fig.2. Variation of cytotoxic activities against *Artemia salina*: a) percent mortality and b) LC_{50} values (±SD) from Cursina III variety and some accessions of *C. xanthorrhiza*. Values followed by the same superscripts are not significantly different (p<0.05) by LSD test.

The cytotoxic activity of ethanol fraction of some accessions were evaluated against brine shrimp for potency preliminary screening of cytotoxic. The results of brine shrimp lethality test are given in Fig. 2. The percentage mortality (lethality) of brine shrimp was found to be directly proportional to the concentration of ethanol fraction in all samples. All samples were found to be toxic (LC_{50}< 1000 µg mL^{-1})[10] and observed not difference significant by LSD test (P<0.05).

As shown in Fig. 3.a, percentage Vero cell mortality was found to be directly proportional with concentrations of ethanol fractions of *C. xanthorrhiza* accessions except in SB accession showed not proportional with concentrations.For values of LC_{50}in Vero cell line (Fig. 3.b),WG accession (0.88 ± 6.72 µg mL^{-1}) showed most cytotoxic followed with Cursina III variety (6.78 ± 6.41 µg mL^{-1}) and accessions of KA (20.75 ± 1.15 µg mL^{-1}), SG (38.00 ± 5.41 µg mL^{-1}) and SB (109.70 ± 6.72 µg mL^{-1}). On the other hand, the cytotoxicity of ethanol fraction of some accessions of *C. xanthorrhiza* on human breast adenocarcinoma (MCF-7) were showed in Fig. 4. Ethanol fraction of Cursina III variety, WG and KA accessions were found to be more effective (not significant at P < 0.05 with LSD test) against MCF-7 with percentage mortality values of 86.3 ± 1.81%, 84.59 ± 2.19%, and 80.04 ± 1.68%, respectively. The ethanol fraction of SB and SG accessions showed

moderate lethality in MCF-7 with mortality of 68.99 ± 1.10 and 56.69 ± 2.17%, respectively. The result showed that ethanol fraction of WG accession had a potency anticancer activity against MCF-7 cell. Curcumin was considered to be responsible for anticancer in MCF-7 cell[12].

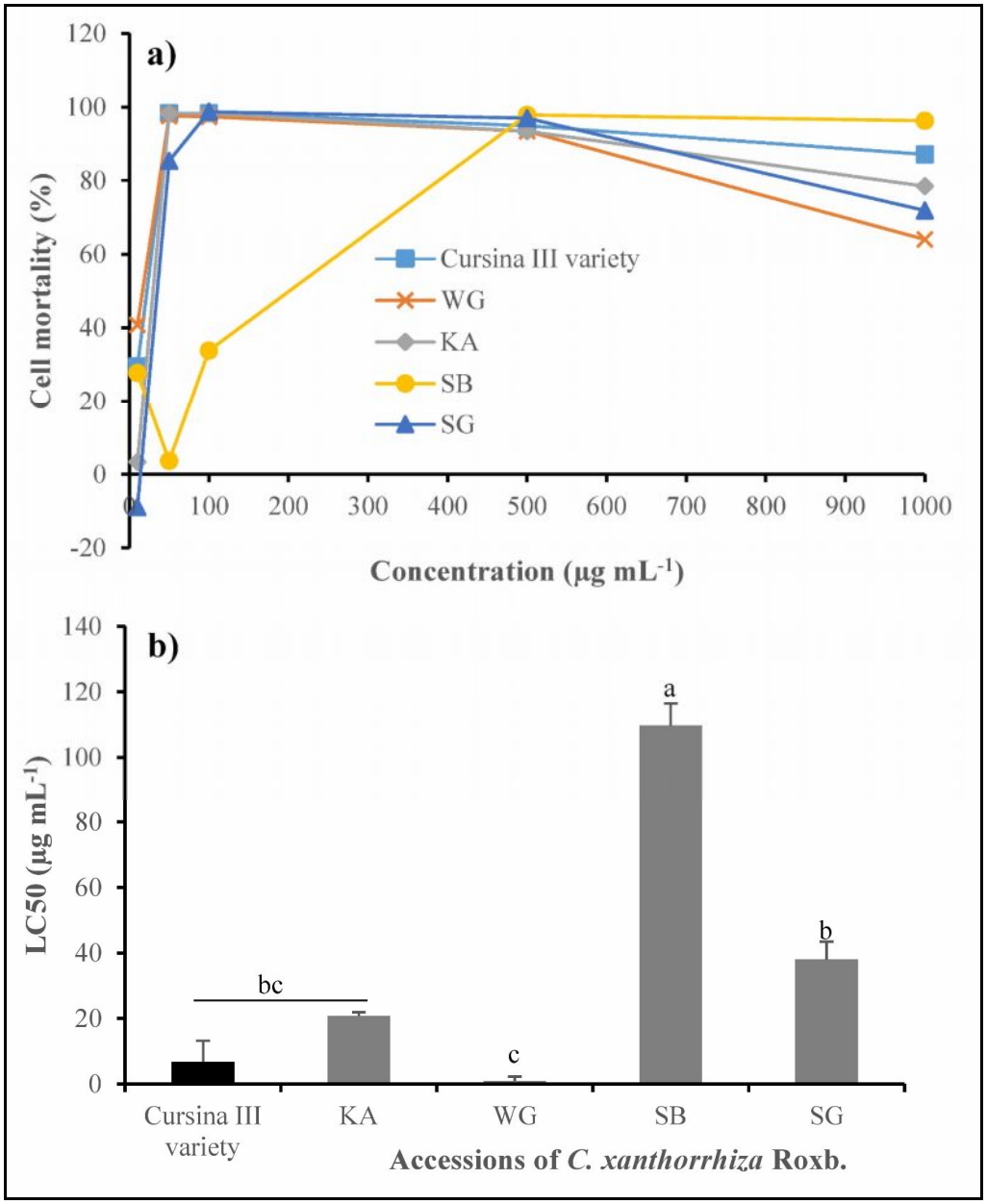

Fig.3. Variation of cytotoxic activities against Vero cell lines: a) percent cell mortality and b) LC_{50} values (±SD) from Cursina III variety and some accessions of *C. xanthorrhiza*. Values followed by the same superscripts are not significantly different ($p<0.05$) by LSD test.

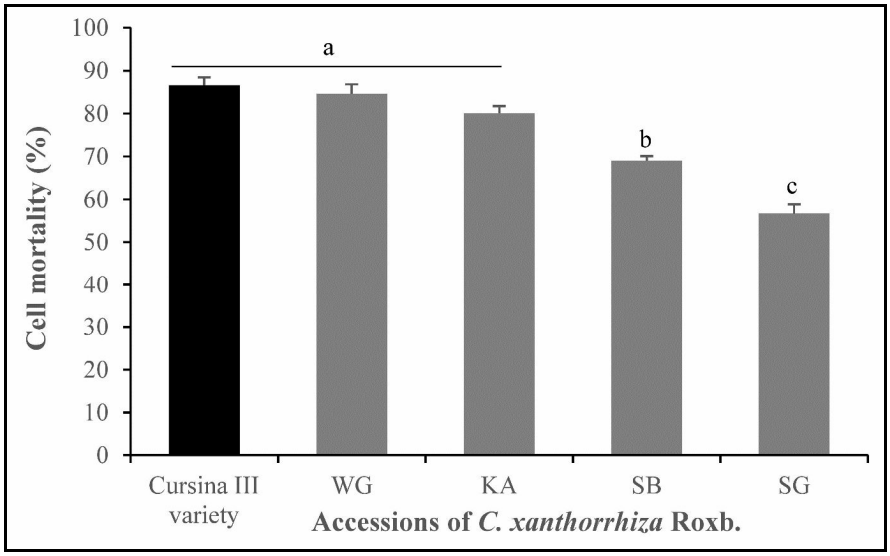

Fig.4. Variation of cytotoxic activities against MCF-7 cell lines in Cursina III variety and some accessions of *C. xanthorrhiza*. Values followed by the same superscripts are not significantly different ($p<0.05$) by LSD test.

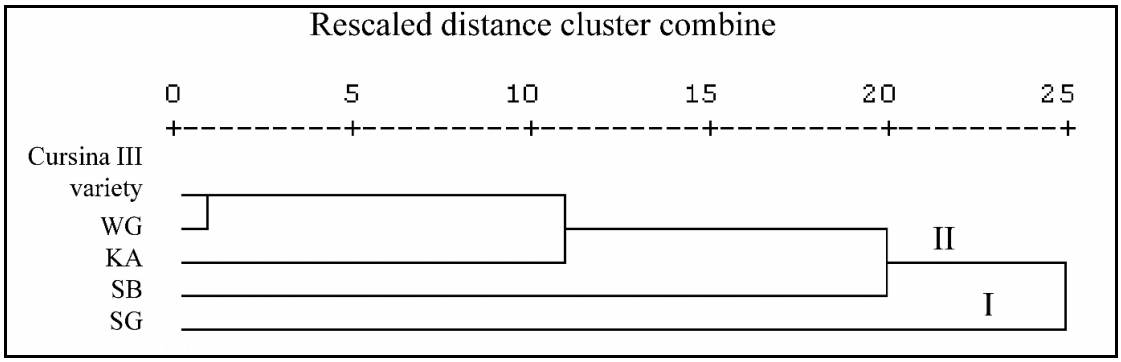

Fig.5. Dendrogram showing the relationship (diversity) among different accession of *C. xanthorrhiza* based on curcumin contents and cytotoxic activities.

Hierarchical cluster analysis based on curcumin contents and cytotoxicity from some accession of *C. xanthorrhiza* into two main groups (Fig. 5). The first group formed by the Cursina III variety, WG, KA, and SB accessions is characterized by high curcumin contents and more effective as cytotoxicity. The second group, formed by the SG accession that characterized low curcumin content and moderate cytotoxic activity. WG accession of *C. xanthorrhiza* identified as promising accession for breeding program to be used high yield curcumin production and anticancer activity.

Conclusion

In conclusion, the WG accession of *C. xanthorrhiza* showed high curcumin contents and cytotoxicity that not differences with the Cursina III variety used as controls. Furthermore, the WG accession can be used as initial material in breeding programs based on bioactive and bioactivity parameters.

Acknowledgments

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