Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

# Xanthorrhizol contents, α-glucosidase inhibition, and cytotoxic activities in ethyl acetate fraction of *Curcuma zanthorrhiza* accessions from Indonesia

Waras Nurcholis[a,b,*], Ahmad Ajruddin Munshif[a], Laksmi Ambarsari[a]

[a] *Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Jawa Barat, Indonesia*
[b] *Tropical Biopharmaca Research Center, Bogor Agricultural University, Jawa Barat, Indonesia*

ARTICLE INFO

*Article history:*
Received 24 September 2017
Accepted 9 November 2017
Available online xxx

*Keywords:*
Accessions
Cytotoxic
Plant breeding
Xanthorrhizol
α-Glucosidase

ABSTRACT

*Curcuma zanthorrhiza* Roxb., Zingiberaceae, a species from Indonesia with xanthorrhizol as the major metabolite, has been used as a folk medicine in several of pharmacological activities. This work aimed to evaluate the xanthorrhizol contents, α-glucosidase inhibition, and cytotoxic activities in ethyl acetate fraction from accessions of *C. zanthorrhiza*. High-performance liquid chromatography investigated xanthorrhizol content with the standard. Pharmacological activities were evaluated by inhibition of α-glucosidase, the brine shrimp lethality test, and anticancer activity. The ethyl acetate fraction yield varied from 8.24% (Karanganyar) to 13.13% (Sukabumi). The xanthorrhizol contents were found to be in the range 43.55% to 47.99% with Ngawi and Wonogiri promising accessions having the lowest and highest value, respectively. $IC_{50}$ value for α-glucosidase inhibition ranged from 339.05 μg/ml (Karanganyar) to 455.01 μg/ml (Ngawi). $LC_{50}$ value for cytotoxic activities ranged from 33.25 μg/ml (Ngawi) to 42.28 μg/ml (Karanganyar) in brine shrimp lethality test, 3.10 μg/ml (Karanganyar) to 9.85 μg/ml (cursina-III) in Vero cell, and 1.17 μg/ml (Ngawi) to 6.83 μg/ml (Sukabumi) in MCF-7 cell. In this study, *C. zanthorrhiza* accessions have a high in xanthorrhizol contents and cytotoxic activities that showed a high potential of studied accessions for breeding programs on a commercial scale.



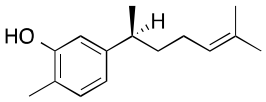
## Introduction

*Curcuma zanthorrhiza* Roxb., also known as Java turmeric (namely "Temulawak" in Indonesia), is a well-known rhizomatous herb that belongs to the Zingiberaceae family (Kim et al., 2014; Nurcholis et al., 2016a). The origin of *C. zanthorrhiza* is Indonesia that distributed in Southeast Asian Region (Suksamrarn et al., 1994; Salea et al., 2014). Moreover, it is grown wild and cultivated widely in Malaysia, Thailand, Sri Lanka and Philippines (Devaraj et al., 2010). It has been traditionally used to overcome various diseases such as stomach diseases, liver disorders, constipation, bloody diarrhea, dysentery, children's fevers, hemorrhoids, and skin eruptions (Hwang et al., 2000). Xanthorrhizol (1), a bisabolane-type sesquiterpenoid compound with IUPAC name of 2-methyl-5-[(2*R*)-6-methylhept-5-en-2-yl]phenol, is the major bioactive constituent contained in rhizomes of *C. zanthorrhiza* (Oon et al., 2015). In the literature, xanthorrhizol of *C. zanthorrhiza* rhizomes are considered to possess anticancer (Kang et al., 2009; Kim et al., 2013), antimicrobial (Rukayadi and Hwang, 2006, 2013; Rukayadi et al., 2006, 2011), anti-inflammatory (Lim et al., 2005; Chung et al., 2007), antioxidant (Lim et al., 2005; Jantan et al., 2012), antihyperglycemic (Kim et al., 2014), antihypertensive (Ponce-Monter et al., 1999; Campos et al., 2000), antiplatelet (Jantan et al., 2008), nephroprotective (Kim et al., 2005), hepatoprotective (Kim et al., 2004; Hong et al., 2005), and estrogenic effect (Anggakusuma et al., 2009). Because of this property, it's important to explore *C. zanthorrhiza* accessions with high xanthorrhizol contents. The yield and biological activities of xanthorrhizol have previously been reported to be affected by geographical location (Nurcholis et al., 2012), but remains unclear whether it was caused by environmental factors or genetic variability. Furthermore, the evaluation of xanthorrhizol contents and pharmacological activities of the different accessions of *C. zanthorrhiza* remains unexplored, and knowledge is limited. α-Glucosidase inhibition and cytotoxic activities of Indonesia accessions have not been tested so far. This knowledge of accessions can develop further insight for *C. zanthorrhiza* breeders to finding new varieties.

* Corresponding author.
*E-mail:* wnurcholis@apps.ipb.ac.id (W. Nurcholis).

https://doi.org/10.1016/j.bjp.2017.11.001

Therefore, in this study to examine yield in ethyl acetate fraction, xanthorrhizol contents, α-glucosidase inhibition, and cytotoxic activities characters without environmental influences, the rhizomes were grown under same environmental and soil conditions, so that the results are comparable and differences should reflect differences between the various accessions of *C. zanthorrhiza* genetically.




1

## Materials and methods

### *Plant material*

The rhizome of four accessions and one variety of *Curcuma zanthorrhiza* Roxb., Zingiberaceae, in 2013 from different Indonesian locations were collected. Variety of *C. zanthorrhiza* namely Cursina-III that received from Indonesian Spices and Medicinal Crops Research Institute. The plant material was identified by Mr. Topik Ridwan, and voucher specimens have been deposited at Tropical Biopharmaca Research Center, Bogor Agricultural University (BMK2013080001-BMK2013080005). Sampling locations and their geographic coordinates are shown in Box 1. Rhizomes sample of *C. zanthorrhiza* were planted at the experimental site of SOHO Centre of Excellent in Herbal Research, Sukabumi, West Java, Indonesia (6°49′55.49″ S, 106°49′3.09″ E; average altitude of 1697 m) in October 2013. The cultivation was arranged in a completely randomized design with three replications. All rhizomes sample were grown under the same conditions with 50 cm × 60 cm spacing and fertilized with 20-ton manure $ha^{-1}$ which given one month before planting. The rhizomes of plants were harvested at the nine months after planting (in June 2014). The rhizomes samples were cut, dried and powdered. The powder was stored at room temperature until the extraction.

### *Xanthorrhizol extraction*

The extraction was performed by maceration method according to Hwang et al. (2000) with modification. Briefly, the powdered rhizomes (25 g) were extracted with 75% (v/v) methanol (250 ml) at room temperature for 24 h and then filtered using Whatman paper filter No. 4. The methanol extract was concentrated by evaporation (Buchi, R-250, Switzerland) at 50 °C. These extracts were then fractionated with water:ethyl acetate in a ratio of 1:1 (v/v). The ethyl acetate fraction was separated and then concentrated by rotary vacuum evaporator (Buchi, R-250, Switzerland) at 50 °C. These extracts of ethyl acetate fraction were recorded as yield and stored at 4 °C until analysis.

### *Xanthorrhizol analysis*

The xanthorrhizol content in rhizomes sample of the ethyl acetate fraction was determined by HPLC using a xanthorrhizol standard which was isolated from the methanol extract of *C. zanthorrhiza* rhizome with purity 85.42% by HPLC analysis (Nurcholis et al., 2012). All solvents used were HPLC grade. Briefly, 50 mg of sample fraction was dissolved in 25 ml of ethanol by sonication for 1 h at room temperature. After filtration through a 0.45-μm membrane filter, an amount of 20 μl sample solutions were injected into HPLC system. HPLC analysis was performed using a system of LC-20A series (Shimadzu, Tokyo, Japan) with system equipped a diode array UV–vis detector. Chromatographic separation was achieved by using a Phenomenex C18 column (150 mm × 4.6 mm ID, 5 μm particle size) with column oven temperature at 40 °C. The mobile phase used consist of 0.001% formic acid in water (A) and methanol (B) with gradient elution program of 90–10% (A) for 0–12 min and 90% (A) for 13–17 min. Elution was carried out at flow rate 1 ml/min and monitored at 224 nm for quantitation of xanthorrhizol. Standard stock solutions of xanthorrhizol were prepared in methanol at concentrations of 200 μg/ml. Results were obtained by comparing with the standard of xanthorrhizol and then were expressed as a percentage (w/w) extract to weight basis.

### *α-Glucosidase inhibition analysis*

The α-glucosidase inhibition of ethyl acetate fraction in samples was analyzed according to the method reported by Mayur et al. (2010). In brief, 10 μl sample of different concentrations was a mixture with 50 μl of 0.1 M phosphate buffer (pH 7.0), and 25 μl of 0.5 mM pNPG. This mixture reaction was added 25 μl of a α-glucosidase solution (0.2 Unit/ml) and incubated at 37 °C for 30 min. Before reading of the absorbance at 410 nm with a microplate reader (Epoch Biotech, USA), the enzymatic reaction was stopped by adding 100 μl of 200 mM $Na_2CO_3$. The inhibition activity was expressed as percentage inhibition of enzyme activity. The inhibition curves of α-glucosidase in different concentrations were prepared, and $IC_{50}$ values were obtained.

### *Cytotoxic analysis*

Screening of preliminary cytotoxic activity in ethyl acetate fraction of rhizomes sample (in the concentration of 10–200 μg/ml) was analyzed using the brine shrimp lethality test (BSLT) according to the general procedure described by Meyer et al. (1982). Cytotoxic activities were measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma–Aldrich, St. Louis, MO, U.S.A) colorimetric assay according to the method by Handayani et al. (2013) with some modification. MCF-7 cancer cell line (ATCC HTB 22) and Vero non-cancerous cell line (ATCC CCL 81) were used cytotoxic analysis, and there were obtained from Primate Research Center, Bogor Agricultural University. Cell lines were cultured in Dulbecco's minimum Eagle's medium (Gibco, Rockville, MD, U.S.A.) with supplemented with fetal bovine serum (10%; Sigma–Aldrich, St. Louis, MO, U.S.A), 100 μg/ml penicillin (Gibco, Rockville, MD, U.S.A.) and 100 μg/ml streptomycin (Gibco, Rockville, MD, U.S.A.). In brief, $2 \times 10^{-3}$ cells/ml were exposed to different rhizomes sample concentration of 10–500 μg/ml in Vero cell and 3.75–60 μg/ml in MCF-7 cell for 72 h. The untreated cells as the control group were also included. After treatment, the medium was removed, and cells were incubated with 20 μl MTT (2 mg/ml). After 4 h incubation, 100 μl HCl-isopropanol (0.1 N) was added to the reaction mixture. Finally, the absorbance was measured with a microplate reader (Bio-Rad 680, USA) at 595 nm. The mortality percentage curves were prepared, and $LC_{50}$ values were obtained.

### *Statistical analysis*

All data were subjected to statistical analysis using Statistical Tool for Agricultural Research software version 2.0.1. Differences between the accessions were performed by Least Significant Difference (LSD) test. The yield in EA fraction, xanthorrhizol content, α-glucosidase inhibition, and cytotoxic activities traits of samples were used to determine the relationship between the different accessions of *C. zanthorrhiza* by cluster analysis using the Minitab 16 software. Euclidean distance was selected as a measure of similarity and the single linkage method was used for cluster dentition.

Please cite this article in press as: Nurcholis, W., et al. Xanthorrhizol contents, α-glucosidase inhibition, and cytotoxic activities in ethyl acetate fraction of *Curcuma zanthorrhiza* accessions from Indonesia. Revista Brasileira de Farmacognosia (2017), https://doi.org/10.1016/j.bjp.2017.11.001