Acute Toxicity of Ethanol Extract of *Curcuma zedoaria* Rosc (Zingiberaceae) Rhizomes on Brine Shrimp Larvae and Zebrafish Embryos

Gustini Syahbirin[1]*, Kurnia Alysia Aditianingrum[2], Kusdiantoro Mohamad[3]
[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia, [2]Undergraduate Study Program of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia, [3]Division of Anatomy Histology and Embryology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia.
*Corresponding author: gustinisy@apps.ipb.ac.id

Abstract

Curcuma zedoaria Rosc (Zingiberaceae), known as white turmeric or temu putih, is commonly used as traditional medicine in Indonesia. The toxicity of this herb needs to be studied to improve its application. This study aimed to evaluate the toxicity of the crude ethanol extract of temu putih rhizome against brine shrimp (*Artemia salina*) larvae and zebrafish (*Danio rerio*) embryos and to analyze the potential active compounds contained in the extract. The crude ethanol extract showed a 50% lethal concentration value (LC_{50}) at 588 ppm against brine shrimp larvae and 224 ppm against zebrafish embryos. Based on the gas chromatography-mass spectrometry analysis, the suspected active compounds that play a role in the toxicity were epicurzerenone, curzerene, and curzerenone, while 2,4,6-trimethylacetophenone was the predominant compound.

Keywords: acute toxicity, *Artemia salina*, *Curcuma zedoaria*, *Danio rerio*, LC_{50}

Received: 24 September 2023 **Revised:** 15 December 2023 **Accepted:** 16 January 2024

INTRODUCTION

Indonesia has various types of nutritious plants, one of which comes from the Curcuma genus. The Curcuma genus has been widely studied and utilized, including *C. longa*, *C. zedoaria*, *C. sylvatica*, *C. aeruginosa*, *C. amada*, *C. aromatica*, *C. brog*, *C. caesia*, and *C. rakhtakanta* (Angel *et al*., 2012). The white turmeric plant (*C. zedoaria*) is distributed throughout most of Southeast Asia and the Himalayas, India (Sirirugsa, 1999). Heyne (1987) reported that white turmeric grows wild in Indonesia on Mount Dempo, South Sumatra, as well as in the teak forests of East Java.

The white turmeric rhizome is white and pale yellow and has a bitter taste. In traditional medicine, white turmeric rhizomes are consumed directly or in the form of herbal medicine (Rahman *et al*., 2013; Hisyam *et al*., 2023). The benefits of white turmeric as a medicine have been widely studied, including anticancer (Radji *et al*., 2010), anti-inflammatory (Kaushik and Jalalpure, 2011), antioxidant (Angel *et al*., 2012; Sumathi *et al*., 2013), antidiarrhea (Nuratmi *et al*., 2006), and antifungal (Cristiane *et al*., 2011; Hidayah *et al*., 2020). Kim *et al*. (2005) reported that water extract of white turmeric rhizomes could be used as hepatoprotective. The alkaloid, phenolic, flavonoid, saponin, glycoside, and steroid compounds contained in the methanol extract of white turmeric rhizomes are efficacious in preventing the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sumathi *et al*., 2013).

Jang *et al*. (2001) isolated three active compounds from the ethyl acetate fraction which were proven to be anti-inflammatory, namely 1,7-bis(4-hydroxyphenyl)1,4,6-heptatriene-3-one, procurcumenol, and epiprocurcumenol. Rahman *et al*. (2013) succeeded in isolating curzerenon andalismol, which were proven to be able to inhibit the growth of cancer cells. Paramapojn and Gritsanapan (2009) reported that crude ethanol extract of white turmeric rhizomes contains curcuminoids consisting of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcuminoids can

inhibit OVCAR-3 ovarian cancer cells (Syu *et al*., 1998).

Acute toxicity shows the degree of toxic effect of a compound that occurs within a short time after administration in a single dose or repeated administration within a limited time with a maximum time limit of 14 days (generally 24 hours) (Syabana, 2010; Hamid *et al*., 2019). The acute toxicity test that is widely used is the brine shrimp lethality test (BSLT) using *Artemia salina* larvae as test animals. This method can be used as an initial screening for the content of active compounds contained in plant extracts. This test has several advantages, including being relatively cheap, fast, and positively correlated with the cytotoxic power of anticancer compounds (Meyer *et al*., 1982; Zakwan *et al*., 2023).

The zebrafish embryo toxicity test (ZFET) is an advanced screening stage of BSLT to determine the toxic power of active compounds in plant extracts. Zebrafish (*Danio rerio*) have several advantages, such as developing simultaneously, transparent morphology, being permeable to drugs, and can be easily manipulated using genetic and molecular approaches (Kari *et al*., 2007). The results of toxicity tests on zebrafish embryos have been shown to have a positive correlation with the results of toxicity tests on mammals (Parng *et al*., 2002). In vivo testing of anticancer compounds in embryos and zebrafish has been widely reported (Berghmans *et al*., 2005; Moore *et al*., 2006; Nicoli and Presta, 2007).

Traditional medicines must require safe criteria according to the requirements set by the National Food and Drug Supervisory Agency (BPOM). Based on BPOM Decree Number HK.03.1.23.06.10.5166 of 2010 (BPOM, 2010), the permitted solvent for extracting herbal medicines is ethanol (ethyl alcohol, C_2H_5OH). Regarding its application as an herbal medicine, this study was limited to testing crude extracts using ethanol as a solvent. This study aimed to evaluate the toxicity of crude ethanol extract of white turmeric rhizomes using the BSLT and ZFET methods, as well as analyzing the classes of compounds contained therein using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Ethical Approval

This study followed the principles of use and animal welfare principles and has received approval from the Animal Ethics Commission, SVMBS, IPB University with Number 016/KEH/SKI/XI/2014.

Study Period and Location

Zebrafish embryo toxicity test was performed in the Laboratory of Embryology, School of Veterinary Medicine and Biomedical Sciences (SVMBS), IPB University, Bogor. White turmeric rhizomes obtained from the Biofarmaka garden, Cikabayan, IPB University, Bogor, Indonesia. White turmeric plants were determined at the Bogoriense Herbarium, Biological Research Center, LIPI, Cibinong, Bogor. All procedure for extraction and determination of water content was performed in the Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor. This study performed from October 2014–February 2015.

Rhizome Samples

White turmeric rhizomes were rinsed using water and then sliced with a thickness of 5–7 mm. The samples were air-dried for two days and then dried in an oven at 50°C for 24 hours. After drying, the samples were sieved using an 80-mesh sieve.

Extraction (Radji *et al*., 2010)

White turmeric rhizome powder was macerated in 80% ethanol (1:6) for 3 hours using a water bath shaker at 45°C. The filtrate was separated and evaporated using a rotary evaporator apparatus. The concentrated extract was then freeze-dried. The concentrated extract obtained was weighed, and the yield was calculated using the following equation:

$$\text{Extract yield (\%)} = \frac{\text{A}}{\text{B} \times (1 - \text{water content})} \times 100\%$$

A: Extract weight (g)
B: Initial sample weight (g)

Determination of Water Content (AOAC, 2006)

The porcelain cup was dried in an oven at 105°C for 60 minutes, then cooled in a desiccator for 30 minutes, and the empty weight was weighed. A total of 3 g of sample was put into the cup and dried in an oven at 105°C for 6 hours. After that, the cup was cooled in a desiccator for 30 minutes and then weighed until a constant weight was obtained. This water content determination was carried out in 3 repetitions (triple) and calculated using the formula:

$$Water\ content\ (\%) = \frac{\text{A} - \text{B}}{\text{C}} \times 100\%$$

A: Weight of cup + sample before drying (g)
B: Weight of cup + sample after drying (g)
C: Initial weight of sample (g)

Toxicity Test on *A. salina* Larva (Meyer *et al*., 1982)

The standard solution was made in a concentration of 4000 ppm with the addition of 100 µL DMSO to help dissolve the extract. Then, the solution was diluted with seawater to obtain final concentrations of 250, 500, 1000, 1500, and 2000 ppm. A total of 10 brine shrimp larvae put in 1000 µL seawater and a certain amount of extract solution into a 4000 µL vial. The mixture was incubated for 24 hours. Death determination was observed microscopically, and brine shrimp on the bottom of the plates with an absence of movement for 5 min were regarded as dead. Repetition was carried out three times. The 50% lethal concentration (LC_{50}) value was determined using a relationship curve between the logarithm of the extract concentration (x-axis) and the probit value (y-axis).

Toxicity Test on Zebrafish Embryos (OECD, 2013)

This study began with obtaining zebrafish eggs from local fish breeders in the Pakansari area, Cibinong, Bogor Regency. The eggs obtained were selected and then placed in 24-well plates. One embryo (4–5 h post-fertilization) per well was placed randomly.

The extract was dissolved in water, and 20 µL/100 mL of DMSO was added (for the dissolution process) to obtain final extract concentrations of 100, 200, 400, 600, and 800 ppm. Five 24-well plates were prepared, each containing 20 embryos as treatment at each concentration and four embryos as an internal control. One 24-well plate was provided for the negative control and another 24-well plate for the DMSO solvent control, each plate containing 20 embryos and four embryos as an internal control. Next, the plates containing zebrafish embryos were placed at room temperature (26 ± 0.5°C) and observed every 24 hours up to 96 hours (four days) using a stereo microscope (Olympus SZX7, Japan) connected to a computer and photo camera.

Observations made include life, death, and hatching ability (hatching, ≥ 48 hours) of an embryo. Embryo lethality was determined based on apical observation of each tested embryo including coagulation of embryos, lack of somite formation, non-detachment of the tail, and lack of heartbeat (OECD, 2013). All observation was performed using a light inverted microscope. The LC_{50} value was determined from the viability value (live-dead) using a log concentration curve (x-axis) with the probit value (y-axis). Meanwhile, hatchability was calculated based on percentage (%).

Analysis with HPLC Instruments (Jayaprakasha *et al*., 2002)

HPLC analysis was carried out at the IPB Biopharmaceutical Study Center, Taman Kencana, Bogor. The Hitachi L-2420 HPLC instrument is equipped with an ultraviolet-visible (UV-Vis) detector with a wavelength of 425 nm and a C18 column. The mobile phase used consisted of 2% acetic acid (v/v) and acetonitrile

with a flow rate of 1 mL/minute. Gradient elution system with a composition of 45–65% acetonitrile and 2% (v/v) 35–55% acetic acid.

Component Identification using GC-MS

GC-MS analysis was carried out at the Forensic Laboratory Center (Puslabfor Polri), National Police Headquarters, South Jakarta. The sample was injected into an Agilent 19091S-436 GC-MS with an HP-5MS capillary column measuring 60 m × 0.25 µm and helium carrier gas at a flow rate of 1.0 mL/min. The maximum column temperature is 350°C, the oven temperature is 100°C for 5 minutes, then increased to 250°C at a rate of 15°C/minute. The processing time is 45 minutes, and the solvent delay time is 5 minutes. The split injector temperature is 290°C with a flow rate of 104 mL/min, pressure of 34,575 psi, injection volume of 1 µL, ionization energy of 69,922 eV, and molecular weight range of 5–8 m/z. Compound identification was carried out by comparing peaks and mass spectra with the Wiley 9TH database.

RESULTS AND DISCUSSION

Determination of Plants, Water Content, and Extract Yield

The plant used in this study was proven to be white turmeric with the scientific name *Curcuma zedoaria* (Christm.) Roscoe and the part studied was the rhizome (based on plant determinations carried out at the Bogoriense Herbarium, LIPI Cibinong Biology Research Center, Bogor). The water content of white turmeric rhizomes was measured at 6.19%, which was used as a correction factor and resulted in a yield of 14% (from 150 g of rhizomes). Physical damage due to uncontrolled water levels can cause contamination, such as microbes. Therefore, the water content of the sample must be measured so that the appropriate storage method can be known to avoid the influence of microbial activity. This sample can be stored to avoid being affected by microbial activity if the water content is less than 10% (Winarno, 1992).

Extract Toxicity to Brine Shrimp Larvae and Zebrafish Embryos

The ethanol extract of white turmeric rhizomes had an LC_{50} value of 588 ppm for the BLST test and 224 ppm for the ZFET test (Table 1). These two LC_{50} values are less than 1000 ppm, thus indicating the toxic nature of the ethanol extract of white turmeric rhizomes (Juniarti *et al*., 2009). Lower LC_{50} values were reported by Akter *et al*. (2012), with the LC_{50} value of crude ethanol extract obtained at 146 ppm. Differences in extraction method and time likely cause this difference because Akter *et al*. (2012) soaked the samples at room temperature for seven days. Apart from that, differences in where plants grow also allow for different secondary metabolite contents so that they have different toxicity.

A toxicity test using zebrafish embryos has been developed as an acute toxicity test to explore new drugs from natural compounds (Kari *et al*., 2007). Even though zebrafish are a more complex organism than brine shrimp larvae, the LC_{50} value obtained is smaller (more toxic) than the BSLT results. This difference is likely caused by the BSLT observation period which is only 24 hours. In contrast, ZFET requires 96 hours of observation so that the active compounds in the extract accumulate in the organs of zebrafish embryos. In addition, with the BSLT method, test animals can only be determined whether the test animal is alive or dead. Meanwhile, using the ZFET method, zebrafish embryos can only be declared dead if they meet one or more of the four existing criteria, namely coagulation, no somite formation, attachment to the tail, and no heartbeat (OECD, 2013).

The crude ethanol extract of white turmeric rhizomes was observed to have a particular effect on test animals. Exposure for 24 hours at low concentrations (100 and 200 ppm) accelerated the embryo hatching process compared to the negative control (Table 2, Figure 1B-C). At a concentration of 100 ppm, the number of embryos increased sharply from 10% at 24 hpf to 85% at 48 hpf. The same thing was observed at a concentration of 200 ppm, and an increase occurred from 45% at 24 hpf to 90% at 48 hpf. This result showed that white turmeric has a

Table 1. LC_{50} value of white turmeric extract on brine shrimp larvae and zebrafish embryos

Acute toxicity test	LC_{50} value (ppm)	Significant level (R^2)
Brine shrimp larva (BSLT)	588	0.98
Zebrafish embryo (ZFET)*	224	0.87

*96 hours post fertilization.

Table 2. Percentage of the number of zebrafish embryos that lived, died, and hatched when given crude ethanol extract of white turmeric rhizomes

Observation	Time (hpf)	Concentration Groups						
		C	SC	Z1	Z2	Z3	Z4	Z5
Lived (%)	24	100	100	100	100	95	80	60
	48	100	100	100	100	5	0	0
	72	100	100	95	95	5	0	0
	96	100	100	90	95	5	0	0
Died (%)	24	0	0	0	0	5	20	40
	48	0	0	0	0	95	100	100
	72	0	0	5	5	95	100	100
	96	0	0	10	5	95	100	100
Hatched (%)	24	0	0	10	45	5	0	0
	48	100	85	85	90	5	0	0
	72	100	100	95	95	5	0	0
	96	100	100	90	95	5	0	0

hpf: hours postfertilization, C: negative control, SSC: solvent control, white turmeric extract concentration Z1: 100 ppm, Z2: 200 ppm, Z3: 400 ppm, Z4: 600 ppm, Z5: 800 ppm.

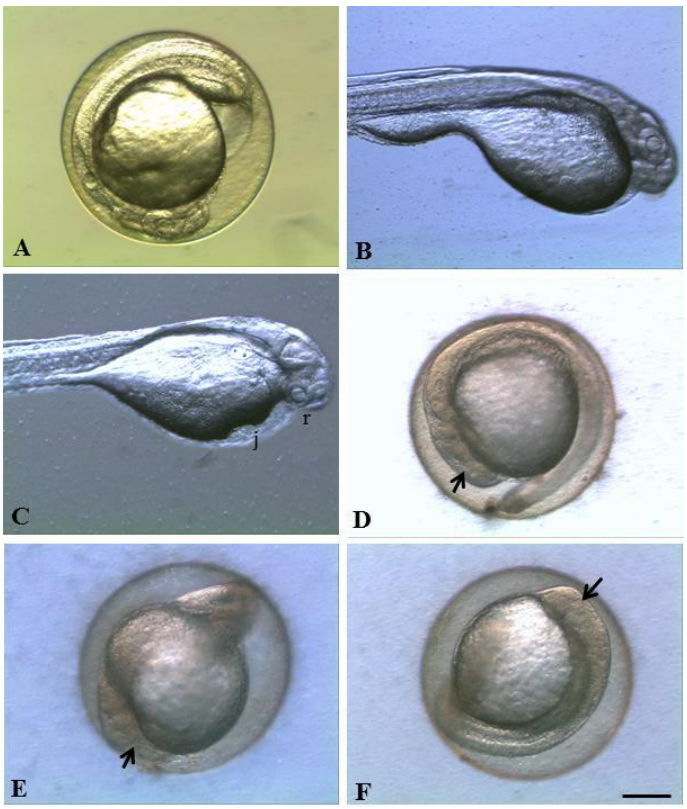

Figure 1. Toxicity test of crude ethanol extract of ginger on 24 hpf zebrafish embryos. (A) control, (B) 100 ppm, (C) 200 ppm, (D) 400 ppm, (E) 600 ppm, (F) 800 ppm treatment. B, C: embryo hatches, j: abnormalities in the heart, r: abnormalities in the jaw, arrows: abnormalities in the head area.

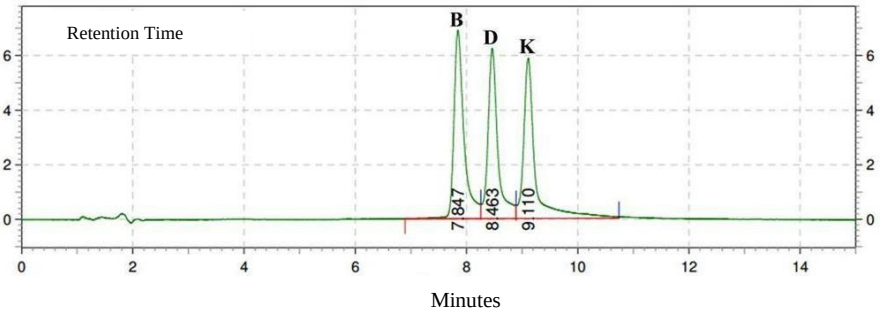

Figure 2. HPLC chromatogram of standard curcuminoids. B: bisdemethoxycurcumin, D: demethoxycurcumin, K: curcumin.

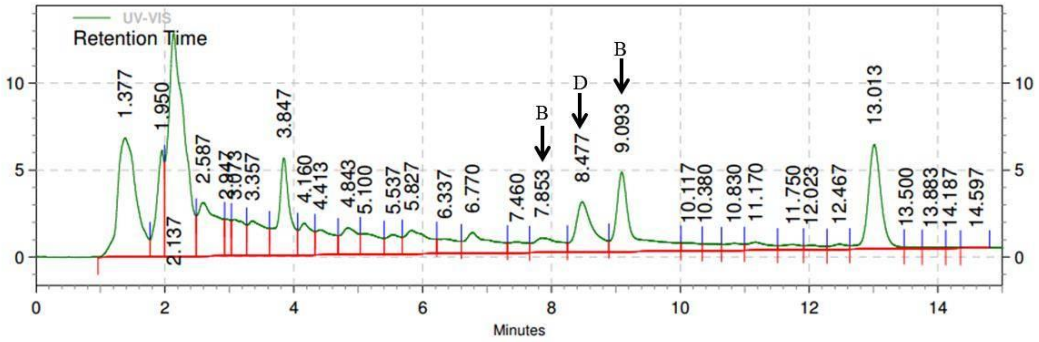

Figure 3. HPLC chromatogram of crude ethanol extract of white turmeric rhizome. B: bisdemethoxycurcumin, D: demethoxycurcumin, K: curcumin.

Table 3. Curcuminoid levels using HPLC instruments

Curcuminoid	Peak area	Standard peak area	Concentrations ($\times 10^{-3}$ mg/g)
Bisdemethoxycurcumin	93746	345871	2.6783
Demethoxycurcumin	219256	305170	7.0995
Curcumin	315534	336042	9.2784
Total			19.0562

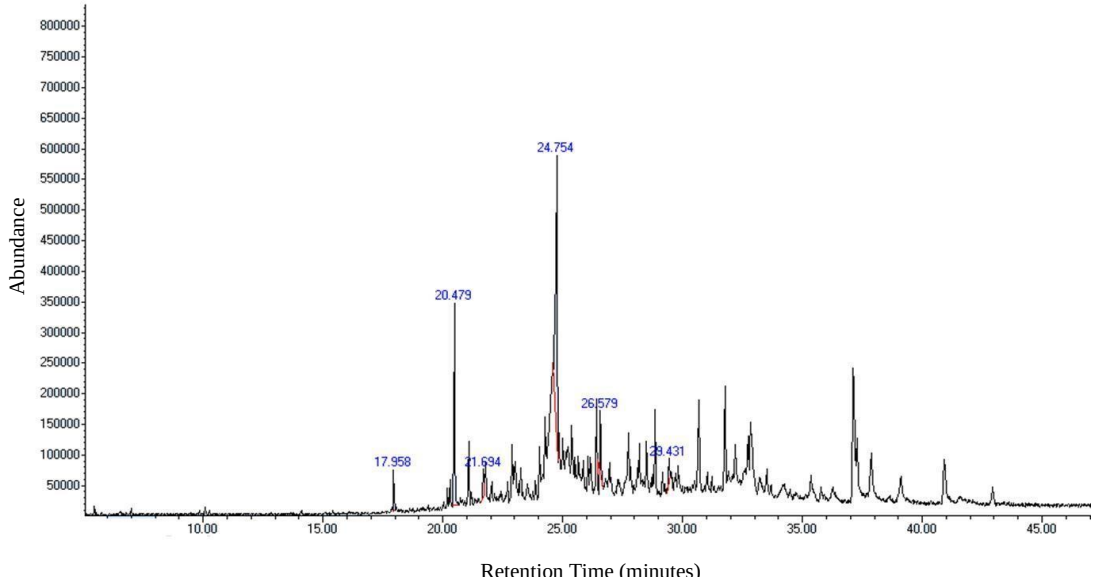

Figure 4. GC-MS chromatogram showed abundance and retention time of compounds in crude ethanol extract of white turmeric rhizome.

Table 4. Compounds in selected crude ethanol extracts of white turmeric rhizomes based on retention time, area, and similarity of compounds

Compounds	Retention time (minute)	Area (%)	Similarity (%)
Curzerene	17.96	0.70	95
Epicurzerenone	20.48	4.01	86
Curzerenone	20.48	4.01	72
β-eudesmol	21.69	1.11	99
2,4,6-trimethylacetophenone	24.76	14.89	38
Calamenene	26.58	2.24	93
Linderazulene	29.43	1.00	90

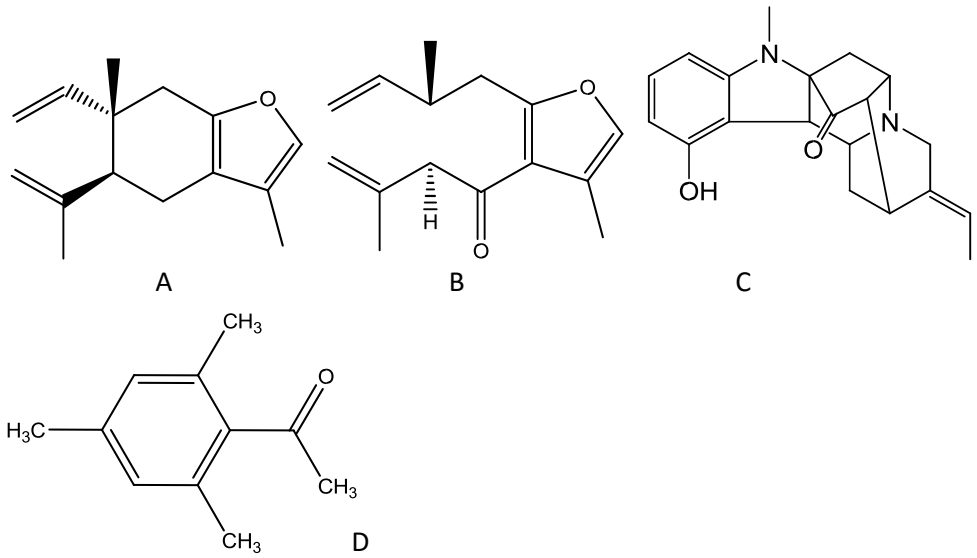

Figure 5. The structures of the compounds curzerene (A), curzerenone (B), and epicurzerenone (C) have the highest similarity (>90%), as well as the compound 2,4,6-trimethylacetophenone (D) with the highest abundance (area 14.89%).

stimulant effect at low concentrations, which can speed up the growth process. However, the effects of this stimulant need to be investigated further, whether it produces normal development or, on the contrary, causes abnormalities.

Apart from the stimulant effect, white turmeric at low concentrations also has a less toxic effect. The less toxic effect is indicated by embryo mortality of 5–10% observed at 72 and 96 hpf at concentrations of 100–200 ppm (Table 2). However, this result showed that at low concentrations (100–200 ppm), the growth stimulant effect in white turmeric is still more dominant than the toxic effect.

The crude ethanol extract of white turmeric rhizomes with higher concentrations (400, 600, and 800 ppm) showed toxic effects (Table 2). At a concentration of 400 ppm, the percentage of embryos that hatched was only 5%. Even at concentrations of 600 and 800 ppm, none hatched. Failure of the embryo to hatch can cause the embryo's death. The extract concentration of 400 ppm, which initially produced a mortality percentage of only 5% at 24 hpf, increased to 95% at 48 to 96 hpf. The most significant percentage of deaths occurred at concentrations of 600 and 800 ppm, with 100% of test animals having died at 48 hpf.

Crude ethanol extract of white ginger rhizomes also causes abnormality in the organs of zebrafish embryos. Some abnormalities occurred in the jaw, heart, and head development (Figure 1C-F).

HPLC Analysis of Crude Ethanol Extract of White Turmeric Rhizomes

The crude ethanol extract content of white turmeric rhizomes was identified using HPLC instruments. Curcuminoid standards were used in the HPLC analysis, each consisting of

bisdemethoxycurcumin, demethoxycurcumin, and curcumin with a concentration of 0.5 ppm (Figure 2). The use of this standard is based on research by Syu *et al*. (1998) and Paramapojn and Gritsanapan (2009), who reported that the crude ethanol extract content of white turmeric rhizomes was mostly curcuminoids.

Based on comparison with standard chromatograms, crude ethanol extract of white turmeric rhizomes contains three types of curcuminoids. Bisdemethoxycurcumin, demethoxycurcumin, and curcumin were detected at retention times of 7.853, 8.477, and 9.093 minutes (Figure 3). Apart from curcuminoids, there were other peaks with higher intensity at a retention time of 1.377, 1.950, 3.847, and 13.013 minutes (Figure 3). However, the compound cannot yet be determined.

The total curcuminoid in the crude ethanol extract of white turmeric rhizomes was only 19.0562×10^{-3} mg/g or 1.9056×10^{-3}%. Curcumin is the most dominant curcuminoid compound with levels of 9.2784×10^{-3} mg/g or 9.2784×10^{-4}% (Table 3). This level is much smaller than that reported by Paramapojn and Gritsanapan (2009). The total curcuminoids in the crude ethanol extract of white turmeric rhizomes from Chiang Mai were reported to be 7.07%, with the dominant compound being demethoxycurcumin (4.92%).

Differences in variety, location, and planting conditions caused curcuminoid content differences in white turmeric rhizomes (Li *et al*., 2011). Apart from that, harvest time also influences the content of active compounds. The harvest age for white turmeric rhizomes used in this study was nine months after planting, while Paramapojn and Gritsanapan (2009) used rhizomes that were 10–12 months after planting. The different harvest age is thought to be what causes low levels of curcuminoids.

The toxicity of the extract, based on the BSLT and ZFET tests, is caused by the presence of active compounds in it. The curcuminoid levels were tiny amount in the samples, so the role of other compounds in the toxicity of the extract needs to be re-examined. Wu *et al*. (2007) reported that curcumin concentrations of around 7.5 µM or the equivalent of 2.8 ppm were able to kill and inhibit the hatching of zebrafish embryos. In this study, the LC_{50} of white turmeric extract was 224 ppm, which contained curcuminoids equivalent to 0.4 ppm. It means far less than the concentration of pure curcumin. Thus, the role of white turmeric extract in acute toxicity is influenced by compounds other than curcuminoids.

In this study, abnormalities observed during the study included the head, heart, eyes, and jaw (Figure 1). Typical abnormalities observed due to exposure to pure curcumin compounds in zebrafish have been reported in the tail fin and shortened body (Wu *et al*., 2007). Meanwhile, abnormalities in the heart and yolk sac are abnormalities that commonly occur in various toxicity test on zebrafish embryos (Syahbirin *et al*., 2017). Thus, it is suspected that other compounds can influence the acute toxicity and inhibition of hatching in zebrafish embryos by the crude ethanol extract of white turmeric rhizomes other than curcuminoids.

GC-MS Analysis of Crude Ethanol Extract of White Turmeric Rhizome

The low levels of curcuminoids in the crude ethanol extract of white turmeric rhizomes as a result of HPLC analysis indicate the suspected presence of other compounds that play a role in its toxicity. Further analysis was carried out using a GC-MS instrument to identify these compounds. The results obtained are shown in Figure 4.

Compounds detected in the chromatogram were selected based on high Qual values (90% similarity) and dominant components (% peak area). A high peak area percentage indicates the dominance of certain compounds in the extract with a specific retention time. The similarity percentage shows how similar the detected compound is to the compound data. Based on the results of GC-MS analysis in Figure 4, there are four compounds with a % similarity of more than 90%, namely β-eudesmol, curzerene, calamenene, and linderazulene. Otherwise, the three compounds have a % similarity of less than 90% but have a high % area, namely 2,4,6-

trimethylacetophenone, epicurzerenone, and curzerenone (Table 4).

Curzerene, epicurzerenone, and curzerenone have been isolated and studied (Mau *et al*. 2003; Rahman *et al*., 2013; Singh *et al*., 2013; Hamid *et al*., 2018). These three compounds (Figure 5A–C) are thought to have toxic effects on the extract, causing death and abnormalities. This is based on study by Mau *et al*. (2003) who reported that curzerene, epicurzerenone, and curzerenone act as antioxidants. Rahman *et al*. (2013) also reported that curzerenone was able to inhibit the proliferation of MCF-7, Ca Ski, and HCT-116 cancer cells. Epicurzerenone and curzerenone are more dominant in the extract compared to curzerene because they have a higher area percentage.

Apart from epicurzerenone and curzerenone, another compound is the most dominant in the extract, with an area percentage of 14.89%. The compound is thought to be 2,4,6-trimethylacetophenone (Figure 5D). The spectrum of this compound has been analyzed for its fragmentation pattern, and the fragmentation pattern has been confirmed with the Spectral Database for Organic Compounds SDBS. The fragmentation of the compound 2,4,6-trimethylacetophenone has been deposited in the Spectral Database for Organic Compounds with accession number 364. However, it is too early to conclude the suspicion of this compound because this compound has a low percentage of similarity, i.e. only 38%. Therefore, further study is needed to determine the existence of this compound and its toxicity to test animals. Testing at low concentrations needs to be continued to determine the impact of specific disorders on zebrafish embryo organs from white turmeric extract or purified active compounds.

CONCLUSION

The crude ethanol extract of white turmeric rhizomes was proven to be toxic using both the BSLT and ZFET tests with LC_{50} of 588 ppm and 224 ppm, respectively. In zebrafish embryos, low concentrations of crude ethanol extract of white turmeric rhizomes have the effect of stimulating embryo hatching, whereas at high concentrations, it causes toxicity and death. Based on HPLC analysis, the levels of curcuminoids detected were small amount, namely 9.2784×10^{-4}%. Based on GC-MS analysis, epicurzerenone, curzerenone, curzerene, and 2,4,6-trimethylacetophenone were detected, with the dominant compounds being 2,4, 6 trimethylacetophenone.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Rector of IPB University, for supporting this study.

AUTHORS' CONTRIBUTIONS

GS and KM: Conceptualization and drafted the manuscript. KAA and KM: Validation, supervision, and formal analysis. GS and KAA: Performed sample evaluation. GS and KM: Performed the statistical analysis and the preparation of tables and figures. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Akter, R., Satter, M. A., Khan, M. S., Rahman, M. S., & Ahmed, N. U. (2012). Cytotoxic effect of five medicinal plants extracts using brine shrimp (*Artemia salina*) test. *Bangladesh Journal of Scientific and Industrial Research*, 47(1), 133–136.

Angel, G. R., Vimala, B., & Nambisan, B. (2012). Antioxidant and antimicrobial activity of essential oils from nine starchy Curcuma species. *International Journal of Current Pharmaceutical Research*, 4(2), 45–47.

AOAC [Association of Official Analytical Chemists]. (2006). Official Method of

Analysis of The Association of Official Analytical of Chemists. Arlington (US): AOAC Int.

Berghmans, S., Jette, C., Langenau, D., Hsu, K., Stewart, R., Look, T., & Kanki, J. P. (2005). Making waves in cancer research: new model in the zebrafish. *Biotechniques*, 39(2), 227–237.

BPOM [Badan Pengawas Obat dan Makanan]. (2010). Peraturan Kepala Badan Pengawas Obat dan Makanan tentang Pencantuman Informasi Asal Bahan Tertentu, Kandungan Alkohol, dan Batas Kadaluwarsa pada Penandaan/Label Obat, Obat Tradisional, Suplemen Makanan dan Pangan. Jakarta (ID): BPOM.

Cristiane, S., Mesquita, S., Bertoni, T. A., Guilhermetti, E., & Svidzinski, T. I. E. (2011). Antifungal activity of the extract of *Curcuma zedoaria* against yeasts of the genus Candida isolated from the oral cavity of patients infected with the human immunodeficiency virus. *Revista Brasileira de Farmacognosia*, 21(1), 128–132.

Hamid, I. S., Ekowati, J., & Purnama, M. T. E. (2019). *Kaempferia galanga* L. Inhibiting Effect on Vascular Endothelial Growth Factor (VEGF) and Cyclooxygenase-2 (Cox-2) Expression on Endothelium of Chorioallantoic Membrane. *Indian Veterinary Journal*, 96(9), 80–82.

Hamid, I. S., Aksono, E. B., Sukmanadi, M., & Purnama, M. T. E. (2018). Antiangiogenesis activity test of tin leaf (*Ficus carica* L.) on the number of blood vessels and VEGF expression of chorioallantoic membrane of embryonated chicken eggs. *European Journal of Oncology Pharmacy*, 1(4), e00007.

Heyne, K. (1987). Tumbuhan Berguna Indonesia. Terjemahan dari: De Nuttige Planten Van Indonesië. Jilid ke-1. Jakarta (ID): Yayasan Sarana Wana Jaya.

Hidayah, N., Puspita, R., & Mujahidah, M. (2020). Effect of *Curcuma domestica* Val Extract on Body Weight, Total of Eosinofils and Basofils in Laying Hens Infected with *Salmonella pullorum*. *Jurnal Medik Veteriner*, 3(2), 230–235.

Hisyam, M. A. M., Yuniarti, W. M., Theresia, S. W., Yudaniayanti, I. S., Lukiswanto, B. S., Sudjarwo, S. A., & Widiyatno, T. V. (2023). The Efficacy of Madeira Vine Leaf Extract Combined with Turmeric Rhizome Extract Gel Topically on Second-Degree Burn Wound in Albino Rats. *Jurnal Medik Veteriner*, 6(3), 402–409.

Jang, M. K., Sohn, D. H., & Ryu, J. H. (2001). A curcuminoid and sesquiterpenes as inhibitors of macrophage TNF-release from *Curcuma zedoaria*. *Planta Medica*, 67(6), 550–552.

Jayaprakasha, G. K., Rao, L. J. M., & Sakariah, K. K. (2002). Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. *Journal of Agricultural and Food Chemistry*, 50(13), 3668–3672.

Juniarti, Osmeli, D., & Yuhernita. 2009. Kandungan senyawa kimia, uji toksisitas (brine shrimp lethality test) dan antioksidan (1,1-diphenyl-2-pikrilhydrazyl) dari ekstrak daun saga (*Abrus precatorius* L.). *Makara Sains*, 13(1), 50–54.

Kari, G., Rodeck, U., & Dicker, A. P. (2007). Zebrafish: An emerging model system for human disease and drug discovery. *Discovery*, 82(1), 70–80.

Kaushik, M. L., & Jalalpure, S. S. (2011). Evaluation of anti-inflammatory effect of ethanolic and aqueous extracts of *Curcuma zedoaria* Rosc root. *International Journal of*

Drug Development and Research, 3(1), 360–365.

Kim, D. I., Lee, T. K., Jang, T. H., & Kim, C. H. (2005). The inhibitory effect of a Korean herbal medicine, Zedoariae rhizome, on growth of cultured human hepatic myofibroblast cells. *Life Sciences*, 77(8), 890–906.

Li, S., Yuan, W., Deng, G., Wang, P., Yang, P., & Aggarwal, B. B. (2011). Chemical composition and product quality control of turmeric (*Curcuma longa* L.). *Pharmaceutical Crops*, 5(1), 28–54.

Mau, J. L., Lai, E. Y. C., Wang, N. P., Chen, C. C., Chang, C. H., & Chyau, C. C. (2003). Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. *Food Chemistry*, 82(4), 583–591.

Meyer, B. N., Ferrigni, N. R., Putman, J. E., Jacobson, L. B., Nichol, D. E., & McLaughlin, J. L. (1982). Brine shrimps: a convenient general bioassay for active plant constituent. *Planta Medica*, 45(5), 31–34.

Moore, J. L., Rush, L. M., Breneman, C., Mohideen, M. A. P. K., & Cheng, K. C. L. (2006). Zebrafish genomic instability mutants and cancer susceptibility. *Genetics*, 174(2), 585–600.

Nicoli, S., & Presta, M. (2007). The zebrafish/tumor xenografit angiogenesis assay. *Nature Protocols*, 2(11), 2918–2923.

Nuratmi, B., Nugroho, Y. A., & Sundari, D. (2006). Efek antidiare jus temuputih (*Curcuma zedoaria* Rosc.) dan temumangga (*Curcuma mangga* Val. Et. Zipp.) pada tikus putih. *Media Litbang Kesehatan*, 16(1), 29–34.

OECD [Organization for Economic Co-operation and Development]. (2013). OECD Guidelines for The Testing of Chemicals No. 236. Fish Embryo Acute Toxicity (FET) Test. Paris (FR): OECD.

Paramapojn, S., & Gritsanapan, W. (2009). Free radical scavenging activity determination and quantitative analysis of curcuminoids in *Curcuma zedoaria* rhizome extracts by HPLC method. *Current Science*, 97(7), 1069–1073.

Parng, C., Seng, W. L., Semino, C., & McGrath, P. (2002). Zebrafish: a preclinical model for drug screening. *Assay and Drug Development Technologies*, 1(1 Pt 1), 41–48.

Radji, M., Aldrat, H., Harahap, Y., & Irawan, C. (2010). Uji sitotoksisitas buah merah, mahkota dewa dan temuputih terhadap sel kanker serviks. *Jurnal Farmasi Indonesia*, 5(1), 41–47.

Rahman, S. N. S. A., Wahab, N. A., & Malek, S. N. A. (2013). In vitro morphological assessment of apoptosis induced by antiproliferative constituents from the rhizomes of *Curcuma zedoaria*. *Evidence-Based Complementary and Alternative Medicine*, 2013, 257108.

Singh, P., Singh, P., Kapoor, I. P. S., Singh, G., Isidorov, V., & Szczepaniak, L. (2013). Chemical composition and antioxidant activities of essential oil and oleoresins from *Curcuma zedoaria* rhizomes, part-74. *Food Bioscience*, 3, 42–48.

Sirirugsa, P. (1999). Thai Zingiberaceae: Species diversity and their uses. *Pure and Applied Chemistry*, 70(11), 1–8.

Sumathi, S., Iswariya, G. T., Sivaprabha, B., Dharani, B., Radha, P., & Padma, P. R. (2013). Comparative study of radical scavenging activity and phytochemical analysis of fresh and dry rhizomes of *Curcuma zedoaria*. *International Journal of Pharmaceutical Science and Research*, 4(3), 10691073.

Syabana, M. A. (2010). Toksisitas akut dan subkronis ekstrak air buah murbei (Morus alba L.) pada tikus Sprague dawley [Tesis]. Bogor (ID): Institut Pertanian Bogor.

Syahbirin, G., Mumuh, N., & Mohamad, K. (2017). Curcuminoid and toxicity levels of ethanol extract of Javanese ginger (*Curcuma xanthorriza*) on brine shrimp (*Artemia salina*) larvae and zebrafish (*Danio rerio*) embryos. *Asian Journal of Pharmaceutical and Clinical Research*, 10(4), 169–73.

Syu, W. J., Shen, C. C., Don, M. J., Ou, J. C., Lee, G. H., & Sun, C. M. (1998). Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. *Journal of Natural Products*, 61(12), 1531–1534.

Winarno, F. G. (1992). Kimia Pangan dan Gizi. Jakarta (ID): Gramedia.

Wu, J. Y., Lin, C. Y., Lin, T. W., Ken, C. F., & Wen, Y. D. (2007). Curcumin affects development of zebrafish embryo. *Biological and Pharmaceutical Bulletin*, 30(7), 1336–1339.

Zakwan, M., Sutriana, A., Nurliana, Ammar, M., & Novianti, A. N. (2023). Toxicity Test of Flavonoid Compounds from Ethyl Acetate Extract of Malacca Leaves with Brine Shrimp Lethality Test. *Jurnal Medik Veteriner*, 6(3), 15–19.

***