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The toxicity of *Meistera chinensis* rhizome fraction by shrimp larvae with BSLT method

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Abstract

Meistera chinensis is a species that belong to the Zingiberacea. In Southeast Sulawesi, a population of *M. chinensis* was found in the Abuki forest of Konawe Regency. Empirically, its consumed as a cooking spice and increase the immunity . The results of previous research *M. chinensis* fruit is efficacious as an antioxidant, anticancer, antimicrobial and antifungal. This study aims to determine the potential for toxicity and the LC50 value of *M. chinensis* rhizome using the BSLT method. The rhizomes were extracted with 96% ethanol and concentrated using a rotary evaporator. Then fractionation was carried out with methanol and ethyl acetate as solvents. The concentration of the rhizome fraction was 7.81 ppm, 15.63 ppm, 32.25 ppm, 62.5 ppm, 125 ppm, 250 ppm, 500 ppm and 1000 ppm. Positive control used of 1.4 mg potassium bichromate and negative control is DMSO. Toxicity was determined based on the mortality of shrimp larvae and calculated by determining the LC_{50} value. The results showed that the LC_{50} values for the methanol, ethyl acetate and positive control fractions of *M.chinensis* rhizome were 252.88 mg/L, 57.17 mg/L, and 6.22 mg/L, respectively. Based on these results, it can be concluded that the *M. chinensis* rhizome contains chemical content that might be responsible for toxicity and classified as medium toxic (≤1,000 mg/L).

INTRODUCTION

Herbal plants have been widely used throughout the world as a form of health care and modern medicine (Bourhia *et al*., 2019). Traditional medicine is clinically effective and preferred because of the minimal side effects compared to synthetic drugs (Tee *et al*., 2021). Utilization of plant secondary metabolites has been widely used to treat various diseases (Musdalipah et al., 2021).

Zingiberaceae is one of the important families and reported for the high biological activity potential that can treat various diseases (Sharifi-Rad *et al*., 2017). Zingiberaceae has the largest species in the world, including in Indonesia. Several new generations of Zingiberaceae have been discovered such as Cinnamomum, Meistera, and Wurfbainia (de Boer *et al.*, 2018). The safety level of Zingiberaceae as a drug is widely known for the non-significant side effects to chronic toxic. Therefore, it is important to know the potential of medicinal plants by advance studies on medicinal plants (Tee *et al*., 2021).

Meistera chinensis is a species belonging to the Zingiberaceae. *M. chinensis* is a local plant that found in Abuki, Konawe, Southeast Sulawesi. Empirically the plant used as a flavor enhancer in food, pain reliever, and increase immunity. The results of research by Musdalipah *et al* (2021) stated that the secondary metabolites of *M. chinensis* fruit consist of terpenoids, saponins, phenolics, steroids, flavonoids and triterpenoids. The total phenolic and flavonoid content were 30.72 ± 1.07 mgGAE/g and 8.02 ± 0.48 mgQE/g, respectively. *M. chinensis* fruit fraction has very strong antioxidant activity with an IC_{50} of 42.7 ± 3.53 mg/L (F8). The toxicity test of fruit fraction using the BSLT method was found to be very toxic with an LC_{50} of 5.2 ± 0.72 mg/L (Musdalipah *et al.*, 2021b).

Toxicity test is the first step in the safety parameters of a drug before it becomes a product used in humans. A plant compound is proven to be safe if it has been through a toxicity test using experimental animals, so it is very important to know its toxic potential through the LC_{50} and the spectrum of its toxic effects. Moreover, toxicity test is to detect the toxic effect of a substance on a biological system and to obtain typical dose response data from the test preparation. The data obtained can be used to provide information about the degree of danger of the test preparation of exposure to humans, so that the dosage can be determined for human safety (Jabbar *et al*., 2019).

Most of the new plants need to be studied scientifically such as standardization, biological activity, and toxicity of each plant material. Besides, toxicity experimental assessment has been used as the standard safety studies with efficacy tests (Pitakpawasutthi *et al*., 2021).One of the methods is the *Brine Shrimp Lethality Test* (BSLT). This method can identify the toxicity of natural materials (Saragih et al., 2020). The BSLT method was carried out by calculating mortality by extracts or isolates of *Artemia salina* Leach shrimp larvae for 24 hours. The results obtained as the value of LC_{50} (Lethal Concentration) is the number of doses that can cause the death of shrimp larvae by 50% after 24 hours (Meyer *et al*., 1982).

Etlingera is one of the genus belonging to the Zingiberaceae family which is widely spread on the island of Sulawesi with many species and interesting for investigate (Chan *et al*., 2011; Lourenço *et al*., 2019). Furthermore, Etlingera genus has the same resemblance to the Meistera genus. Research on the Meistera genus itself is still limited, but it is used locally as traditional medicine.The knowledge of potential toxic effects is very important as an initial parameter of drug safety before being tested on humans. Each compound has the potential for toxicity depending on the dose in the body (Fristiohady *et al*., 2020). Currently, research on the *Meistera chinensis* plant continues to be carried out and developed on plant organs such as rhizomes, stems and leaves. This study aims to determine the potential for toxicity and determine the LC_{50} value of *M. chinensis* rhizome using the BSLT method.

MATERIAL AND METHODS

The rhizomes of *Meistera chinensis* were collected in February 2021 in Konawe Regency, Southeast Sulawesi. The plant has been identified by the National Research and Innovation Agency (BRIN). The specimen has been preserved in the herbarium of the Department of Botany The fresh rhizomes were washed with running water, then cut into small pieces and dried at 40°C and protected from sunlight for 3 days (Musdalipah *et al*., 2021)

Fig 1. Fresh Rhizome of *Meistera chinensis*

Extraction of *M. chinensis*

Meistera chinensis rhizome was obtained in Abuki Village, Konawe Regency, Southeast Sulawesi. The rhizomes are washed, sorted and dried by aerating, then the rhizome is cut into small pieces and mashed using a blender. Preparation of *Meistera chinensis* rhizome extract by maceration method. Its powder was weighed about 3,000 g using a digital scale. The rhizome powder was dissolved with 96% methanol in a closed glass for 72 hours with 22,500 mL of 96% ethanol as solvent. The maceration process was carried out for three days and then filtered and remaceration. The filtrate was collected and concentrated using a rotary vacuum evaporator at a temperature of 50 °C to get a thick extract of 105 g.

Fractionation

A total of 15 grams of ethanol extract of *Meistera chinensis* rhizome was dissolved in 100 mL of ethyl acetate and methanol, respectively. It is put into a funnel and 100 mL of water solvent is added for separation by partitioning the extract and solvent. The separating funnel was shaken vigorously until mixed and waited a few minutes until separated into methanol and ethyl acetate fraction. Then removed from the funnel and stored in a different container.

Brine Shrimp Lethality Test (BSLT)

The BSLT method is used to test the toxicity of a compound or extract obtained from plants (Meyer *et al*., 1982). The test was carried out with 100 L of a solution containing 10-15 larvae of *Artemia salina* into a microwell (Saragih *et al*., 2020). The concentration of the rhizome fraction was 7.81 ppm, 15.63 ppm, 32.25 ppm, 62.5 ppm, 125 ppm, 250 ppm, 500 ppm and 1000 ppm. The microwell was incubated for 24 hours at room temperature 22-29^{o}C. The number of dead larvae was

observed using a binocular microscope (12.5x) then added 100 L of methanol and left for 15 minutes (Karchesy *et al.*, 2016; Tee *et al.*, 2021)). The mortality of shrimp larvae was calculated using the probit formula.

Data Analysis

The data obtained is the number of shrimp larvae mortality. The number of shrimp larvae that died at each level was used to determine the percentage of mortality.

$$\%\ \text{mortality} = \frac{\text{number of dead larvae}}{\text{number of tested larvae}} \times 100$$

Data were analyzed probit in linear regression y = a + bx. Toxicity levels are classified as follows: a) very toxic (LC_{50}=30 g/mL), moderately (LC_{50} >30-1000 g/mL), and low (LC_{50} >1000 g/mL) (Meyer *et al.*, 1982; Musdalipah *et al.*, 2021c).

RESULTS AND DISCUSSION

Extraction and fractination

The prepared *Meistera chinensis* rhizome was extracted with 96% ethanol solvent using the maceration method for 72 hours. Ethanol has semipolar properties so that polar and non-polar compounds can be attracted easily in simplicia. The maceration method is a process of immersing the sample with organic solvents at room temperature (Sarker *et al.*, 2006). The thick extract obtained was ±150 g. Based on the weight of the thick extract of *Meistera chinensis* rhizome, the yield value was 5%. This yield calculation was carried out to determine the equivalence value of each gram of thick extract with simplicial.

Brine Shrimp Lethality Test (BSLT) method was chosen because it does not take a long time, is easy, cheap, accurate and requires a small sample (Pisutthanan et al., 2004). The test animal used was *Artemia salina* because it has a response to chemical compounds similar to mammals, such as DNA dependent RNA polymerase and this organism has a Na+ and K+ dependent ATPase transport system (Riaz *et al.*, 2018). The hatching process of A. salina requires an aerator as a source of oxygen and is carried out at room temperature. Hatching takes 24-48 hours (Al-Enazi, 2018).

The test animals used were 48 hours because they already had a complete digestive tract so they were sensitive to an incoming substance. Shrimp larvae that have hatched and left for 48 hours are ready to be sampled. The samples used were 10 individuals at each concentration (Rasyid *et al.*,2020). The concentrations used in this study were 7.81 ppm, 15.63 ppm, 32.25 ppm, 62.5 ppm, 125 ppm, 250 ppm, 500 ppm and 1000 ppm. This concentration was chosen because it is in accordance with the statement that if the LC_{50} of the tested extract is less than 1000 mg/L it is considered toxic (Oluwaniyi dan Obi, 2018). This research was conducted three times (triplo) to obtain good and accurate data. Observations were made after 24 hours of treatment. The results can be seen in Table 1.

Table 1 showed that the methanol fraction has a high mortality effect compared to the ethanol fraction. This result is shown based on the analysis of the LC_{50} using Mini Tab software, LC_{50} for the methanol fraction is 252.88 mg/L, the ethyl acetate fraction is 957.17 mg/L and 6.22 mg/L for potassium dichromate (positive control). LC_{50} is the concentration at which an extract can cause the death of 50% of test animals (Rasyid and Angraeni, 2020).

Acute Toxicity of *Meistera chinensis* Rhizome Extract

Table 1. Result of the toxicity fractions of *Meistera chinensis* rhizome

Concentration (ppm)	Total of larvae	Methanol fraction			Ethyl acetate fraction			Mortality		% Mortaliy		Log		Probit	
		U1	U2	U3	U1	U2	U3	MF	EF	MF	EF	MF	EF	MF	EF
1000	10	8	8	9	3	6	9	8,33	6,00	83%	60%	3,00	3,00	5,95	5,25
500	10	6	7	7	2	6	5	6,64	4,33	67%	43%	2,70	2,70	5,47	4,82
250	10	3	3	7	1	3	7	4,33	3,67	43%	37%	2,40	2,40	4,82	4,67
125	10	3	3	6	0	2	5	4,00	2,33	40%	23%	2,10	2,10	4,75	4,26
62,5	10	3	3	1	1	1	3	2,33	1,67	23%	17%	1,80	1,80	4,26	4,05
31,25	10	3	3	1	0	2	1	2,33	1,00	23%	10%	1,49	1,49	4,26	3,72
15,625	10	2	2	1	0	2	1	1,67	1,00	17%	10%	1,19	1,19	4,05	3,72
7,8125	10	1	1	1	0	1	1	1,00	0,67	10%	7%	0,89	0,89	3,72	3,52

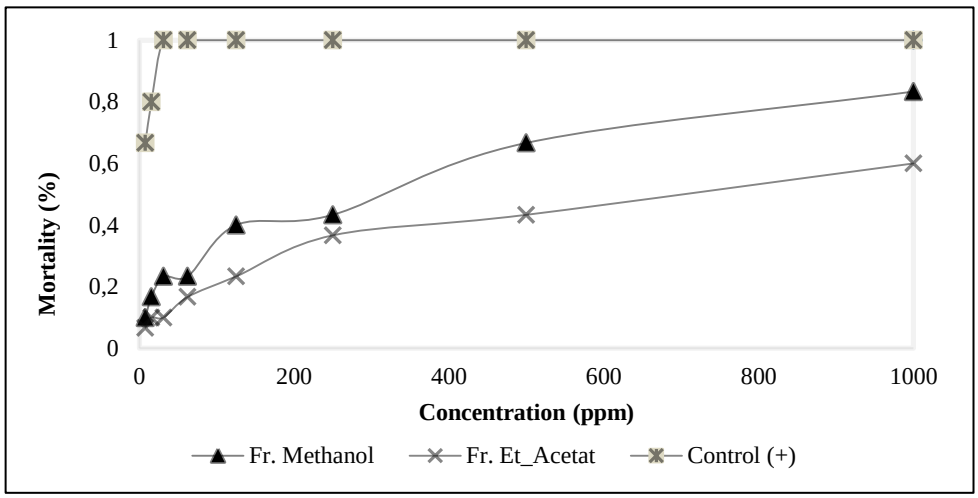

Fig 2. The relationship between the concentration of the *Meistera chinensis* fraction on the mortality of shrimp larvae *A. salina*

In the evaluation of the toxicity of plant extracts with shrimp larvae bioassay, the LC_{50} values ranged from 30-1000 mg/L considered toxic *(*Lachumy *et al*., 2010). Therefore, they can be classified as biologically toxic compounds with pharmaceutical properties. In our study, *Meistera chinensis* rhizome fraction showed significant toxicity to shrimp larvae with an LC_{50} value of 252.88 and 957.17 mg/L (24 hours). Based on the LC_{50} value, the *M. chinensis* rhizome is included in the moderate toxic category where the LC_{50} >30-1000 mg/L. Thus, this indicates that the rhizome of *M. chinensis* may be toxic to *Artemia salina*. The relationship between the concentration of fraction on the mortality of shrimp larvae can be seen in Figure 2.

Figure 2 shows that *Meistera chinensis* fraction is toxic. The fraction that is toxic to shrimp larvae of A. salina is one of the indicators of the biological activity of the bioactive content which is thought to be efficacious as anticancer. Potential as an anticancer is thought to be because the rhizome fraction of *M. chinensis* contains secondary metabolites similar to fruit, such as flavonoids, phenolics and terpenoids (Musdalipah, *et al*., 2021). The results of research in Zingiberaceae are efficacious as antioxidants, toxicity, immunomudolator, anticancer and antibacterial (Haleagrahara *et al.*, 2010; Juwita, *et al*., 2018).

Most of the secondary metabolites in each plant contain phenolic and flavonoid compounds, and have various pharmacological activities (Sungthong and Srichaikul, 2018). Furthermore, one of Zingiberacea that has a similar structure with *M. chinensis* is Etlingera. The results showed that *Etlingera elatior* had chemical content such as tannins, flavonoids, terpenoids, alkaloids, and saponins which were efficacious as anticancer, immunomodulatory, anti-inflammatory, antioxidant, antibacterial and cytotoxic, and tyrosinase inhibition (Chan et al., 2011). Another spesies like *Etlingera alba* rhizome contains chemical compounds that might be responsible for antioxidant activity and the extract classified as medium toxic (Wahyuni *et al*., 2021).

CONCLUSION

The LC_{50} for the methanol and ethyl acetate fractions of *Meistera chinensis* rhizome were 252.88 mg/L and 957.17 mg/L, respectively. It can be concluded that the rhizome fraction has a toxic effect on shrimp larvae (≤1,000mg/L).

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