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Phytochemical and Toxicity Assay of *Meistera chinensis* Fruit Extract: The Endemic Plant of Southeast Sulawesi

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ABSTRACT
Meistera chinensis is one of the endemic plants of Southeast Sulawesi from the Zingiberaceae family which has a large number of species and an interesting potential to be developed as a source medicine. However, there is no report on neither chemical contents nor biological activities of the plant. The aim of this study was to identify the phytochemicals by Thin-Layer Chromatography (TLC) and toxicity of *Meistera chinensis* fruit using the *Brine Shrimp Lethality Test* (BSLT). The fruit of *Meistera chinensis* was macerated with ethanol 95% for 3x24 h, and the solvent was evaporated in a vacuum. The toxicity of the extract was determined by the brine shrimp lethality test use with concentrations of 10, 100, 250, 500, and 750 µg / mL according of death of *Artemia Salina* larvae and calculated by determining the LC_{50} score. The results showed that the phytochemical screening by UV-Vis 254 nm and 366 nm indicates presence phenolics, flavonoids, steroids, terpenoids, alkaloids, and saponins. In the BSLT assay of ethanol extracts of the *Meistera chinensis* fruit were found highly toxic to the *Artemia salina* with IC_{50} values of 5.02 ± 1.11 mg / mL and control 2.26 ± 0.60 mg / mL. These findings indicate that the extract of *Meistera chinensis* fruit is very potential to be used as a medicinal plant and can be developed as a natural anticancer product..

***Keywords**: Toxicity, Meistera chinensis fruit, endemic plant, herbal medicine*

1. INTRODUCTION

Today, herbal plants have been widely used around the world as a form of health care. Herbs play an important role in modern medicine. Medicinal plants sustainably maintain human health for many years [1]. the use of plant secondary metabolites has been widely used to cure various diseases. Traditional medicine is clinically effective and is preferred because it has fewer side effects than drugs of synthetic origin [2,3]. *Zingiberaceae* is an important plant family that has been reported with high potential biological activity that can treat various diseases such as cancer, diabetes, ulcers, and neurological diseases [4].

Zingiberaceae has a large number of species and still requires studies to reveal its chemical content and pharmacological activity. Several new generations were discovered by *Conamomum, Meistera,* and *Wurfbainia* in the newly discovered Zingiberaceae family. Therefore, it is important to determine the medicinal potency of plants by intensifying studies on medicinal plants on an ongoing basis [5].

Meistera chinensis is a species of the *Zingiberaceae* group, which is endemic to Southeast Sulawesi, is widespread, and is very much found in Konawe Regency. Empirically, *Meistera chinensis* is used as a flavor enhancer in food, aches, and increases body immunity. The latest research of *Meistera chinensis* fraction suggest that its has antioxidant and anticancer activity [6].

The safety of potential toxic effects in drug development is important to ensure their use. The low toxicity and clinical effectiveness of natural compounds are one of the aims of the researchers to obtain plant biological activity [6]. Toxicity testing is the first step in drug safety parameters before it becomes a drug product that can be used in humans.

Nowadays, health problems are increasing in line with the development of the disease. The problem of health service costs is increasing, so it is necessary to think about increasing product efficiency

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[7]. The use of herbs as traditional medicine is growing along with increasing exploration data on the types of natural ingredients that can be used to prevent and treat disease [8]. Therefore, from a public health perspective, it is important to know about the recipe and dosage of plants used, especially in terms of toxicity, composition, special properties for patient protection and safety [9]. One of the initial methods for cytotoxic testing is the Brine Shrimp Lethality Test (BSLT). BSLT is a method that is widely used to search for new anticancer compounds derived from plants. The BSLT method has been shown to correlate with anticancer activity. Also, this method is easy to work with, cheap, fast, and quite accurate [10]. In this study, our objective was to investigate, phytochemicals, and toxicity of *Meistera chinensis* fruit using the Brine Shrimp Lethality Test (BSLT).

2. MATERIALS AND METHODS

2.1. Materials

The Plant material was collected in February 2020 in Konawe Regency, Southeast Sulawesi. The plant was authenticated by The Indonesian Institute of Sciences, Indonesian. Voucher specimens have been preserved in the herbarium of The Department of Botany, Indonesian Institute of Sciences. Fresh fruit is washed under running water, then cut into small pieces, then dried at 40 °C and protected from the sun for 4 days.

2.2. Extraction method

The dried sample was refined by using the blender. Refined fruit was weighed approximately 3,000 g then macerated with ethanol 95%, placed on a shaker for 3-5 days while occasionally stirred. Afterward, The extract extract liquid was filtered and evaporated using a rotary evaporator. The crude extract was weighed and obtained 150 g.

2.3. Phytochemicals screening by Thin-Layer Chromatography (TLC)

Phytochemical screening by TLC was used to detect the presence of secondary metabolites such as phenols and tannins, flavonoids, steroids, alkaloids, terpenoids, [11]:

2.3.1. *Test for phenols and tannins.*

0.2 g of the extract were dissolved in 2mL of distilled water and heated using a water bath at 95 °C. then a few drops are added concentrated sulfuric acid followed by a few drops of 5% (w / v) ferric chloride. Tannins are indicated by the formation of a blue, black, green precipitate

2.3.2. *Test for flavonoids.*

0.5 g of the extract was dissolved in 2mL of methanol and heated in a separate water bath. then add a little magnesium powder to the mixture and a few drops of hydrochloric acid afterward. An intense yellow color was formed which turned colorless on the addition of a few drops of diluted acid which indicated the presence of flavonoids

2.3.3. *Test for a steroid.*

About 0,5 gr the crude extract was mixed with 2mL of chloroform and concentrated H_2SO_4 was added sidewise. Steroids are indicated by the presence of a red colour in the layer of chloroform.

2.3.4. *Test for terpenoids.*

About 0,5 gr the crude extract was dissolved in 2mL of chloroform and evaporated to dryness. To this, 2mL of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids.

2.3.5. *Test for alkaloids.*

About 0,5 gr the crude extract was mixed with 2mL of 1% HCl and heated gently. Mayer's reagents were then added to the mixture. The turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.3.6. *Test for saponins.*

Abaout 0.1 g of extract was added. 2mL of distilled water were added separately and stirred in a test tube for 15 minutes. the presence of saponins is indicated by the formation of foam.

2.4. Toxicological Evaluation Brine Shrimp Lethality Assay

The method available by Meyer *et al.* [10] was adopted for testing the toxicity of ethanol extraction. Artemia salina larvae are hatched in a transparent container filled with sea water. Prepared a light bulb with a power of 40-60 watts for hatching at a temperature within 25-30ºC. Oxygen is supplied using a blower. Shrimp larvae were prepared with a 48 hour hatching period. The concentrations used in the BSLT were 10 µg / mL, 100 µg / mL, 250 µg / mL, 500 µg / mL, 750 µg / mL, and negative control. A stock solution was made of 5 mg of extract dissolved in 5 mL of ethanol for 1,000 ppm. The stock solution is pipetteed and put into the vial. Each concentration was repeated 3 times. In the vial, 1% DMSO was added to the air-insoluble fraction. Five milliliters of seawater are added to the vial. All processes were carried out by adding samples to the vial for concentrations of 10 µg / mL, 100 µg / mL, 250 µg / mL, 500 µg / mL, and 750 µg / mL. The control has no samples in it. Ten Artemia larvae were added to each vial. Observations were made for 24 hours. The toxicity test was determined according to the number of death larvae. The toxicity test was assessed by determining the LC_{50} score. To obtain LC_{50}, the mortality rate of larvae after 24 hours of exposure was assessed first.

% mortality= Total larvae mortality/Total larvae x 100%

The toxicity level of a compound is classified according to Mayer [10] classification. LC_{50} score in the range of ≤30 µg/mL is defined as highly toxic. LC_{50} in range of >30–1000 µg/mL is classified as medium toxic, while >1,000 µg/mL as low toxic.

3. RESULT AND DISCUSSION

3.1. Sample preparation and extraction

The preparation of the research sample was made in the form of simplicia and extracted using a recurring maceration method with 96% ethanol as a solvent. The results of the preparation and extraction can be seen in Table 1.

Table 1. Results of preparation, extraction, and yield values of the *Meistera chinensis* fruit extract.

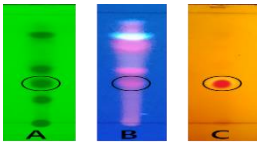
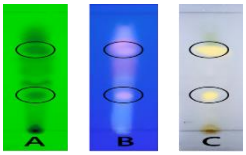
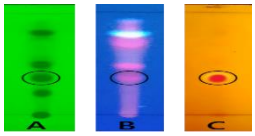
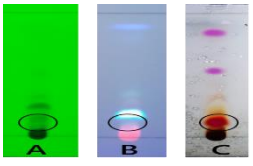
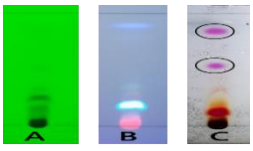
Fresh sample (g)	Dry simplisia (g)	Liquid extract (mL)	extract (g)	Yield (%)
5,000	2,998	22,485	150	5

Based on table 1, the thick extract obtained was 150 g and the yield value was 5%. The yield value was calculated by comparing the weight of the thick extract against the amount of simplicia powder used in the extraction process.

3.2. Identification of a chemical compound by Thin-Layer Chromatography (TLC)

In general, chromatograms are made on silica gel with various types of mobile phases according to the chemical content group as the target of the analysis. Evaluation can be done by documenting photos of the results of a chromatogram with the appropriate reagent. Observations can be made of 254 nm and 365 nm or at other wavelengths that are specific to a known component [12]. The results of the chemical compound can be seen in Table 2.

Table 2. Phytochemical evaluation of the *Meistera chinensis* fruit extract by TLC

No	Identification	Eluent	Reagent	Result of chromatogram **(A=UV 254 nm, B=UV 366 nm, C=Reagent)**	Result
1	Phenolics	*n*-heksan:ethyl acetate (4:6)	$FeCl_3$		+
2	Flavonoids	kloroform:aceton (9:1)	NaOH		+
3	Alkaloids	*n*-heksan:ethyl acetate (7:3)	Dragendroff		+
4	Steroids	*n*-heksan:ethyl acetate (9:1)	H_2SO_4		+
5	Saponins	*n*-heksan:ethyl acetate (9:1)	H_2SO_4		+

(+): Presence; (-): Absence

Based on table 2, phytochemical evaluation, the results showed that *Meistera chinensis* fruit extract contains several secondary metabolites including phenolics, flavonoids, alkaloids, steroids, terpenoids, and saponins. Several secondary metabolites including triterpenoids and flavonoids are known to have anti-cancer properties. Today, medicinal plants of *Zingiberaceae* have become the source of the drug [13]. Plants contain a variety of chemical compounds such as steroids, alkaloids, terpenoids, flavones, phenols, etc that are responsible for many pharmacological properties [14]. In the future, the presence of these metabolites can be used as an indication of the potency of *Meistera chinensis* fruit as a medicine plant.

3.3. *Toxicological Evaluation Brine Shrimp Lethality Assay*

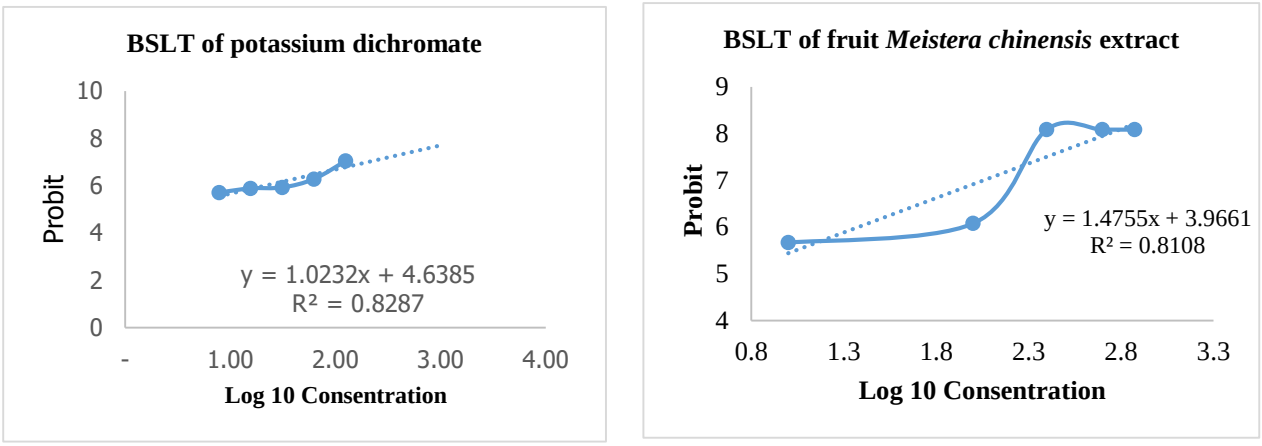
The toxicity of the *Meistera chinensis* fruit extract using the BSLT method. The mortality percentage and LC_{50} of ethanolic extract of *Meistera chinensis* are shown in Table 3.

Table 3. Result of *Toxicological Evaluation Brine Shrimp Lethality Assay* of the *Meistera chinensis* fruit extract

Concentration	Log 10 Kons	replication	Total of Larvae	Larvae mortality	% Mortality	Rate % Mortalitas	Probit	LC_{50} (ppm)
0	0	1	20	0	0	0	0	5.02±1.11
		2	20	0	0			
		3	20	0	0			
10	1	1	20	15	75	75	5.67	
		2	20	16	80			
		3	20	14	70			
100	2	1	20	18	90	86.67	6.08	
		2	20	18	90			
		3	20	16	80			
250	2.31	1	20	20	100	100	8.09	
		2	20	20	100			
		3	20	20	100			
500	2.69	1	20	20	100	100	8.09	
		2	20	20	100			
		3	20	20	100			
750	2.88	1	20	20	100	100	8.09	
		2	20	20	100			
		3	20	20	100			

Based on table 3, toxicity testing of the *Meistera chinensis* fruit extract obtained an LC_{50} value obtained of 5.02 ±1.11 mg/mL and included in the category highly toxic (≤30 ppm). The toxicity level of a compound is classified according to Mayer [9] classification. LC_{50} score in the range of ≤30 µg/mL is defined as highly toxic. Toxicity testing is a common method that can be used to discover new types of drugs. It indicates that substances or chemicals can harm humans or animals. The BSLT technique is a method for identifying the toxicity properties of any chemical [15].

Nowadays, discovering the development of chemicals as an invented method for the production of synthetic drugs, the discovery of new compounds from medicinal plants to treat several diseases is also growing. Generally, the plants of the *Zingiberaceae* family have a secondary metabolism with pharmacological activity. Several studies have reported several medicinal plants that act as cytotoxic. In the last 10 years, various *Zingiberaceae* studies have been continuously developed to find new drug ingredients [15].



(a) (b)

Fig. 1. *Artemia Salina Leach* mortality
(a). Control Positif; (b). Fruit of *Meistera chinensis* extract

Figure 1 shows that each increase in extract concentration, the mortality rate of shrimp larvae increases. The mortality of shrimp larvae by the extract was not much different from the positive control for potassium dichromate (LC_{50} = 2.26 ± 0.60 mg / mL). This means that the higher the concentration of a given substance, the greater the number of shrimp larvae that die. The highest mortality rate occurred at 250 - 750 µg / mL is 100%. In other words, the higher the concentration given, the greater the poisoning it causes. Mortality and survival over some time of exposure are specific effects in acute toxicity tests with long-term exposure.

The mortality rate of larvae was not only caused by the concentration of the fruit *Meistera chinensis* extract given against *Artemia salina larvae*, but also caused by the presence of secondary metabolites of phenolic compounds such flavonoid and phenolic [17]. Polyphenol compound affects the mortality rate of *Artemia salina larvae* Flavonoids as antioxidants, through the mechanism of activating the apoptotic pathway of cancer cells. The mechanism of cell apoptosis in this theory is due to DNA fragmentation. This fragmentation begins with the release of the proximal DNA chain by reactive oxygen compounds such as hydroxyl radicals. Another effect is flavonoids as inhibitors of tumor/cancer proliferation, one of which is by inhibiting protein kinase activity, thereby inhibiting the signal transduction pathway from the membrane to the nucleus cells. Flavonoids inhibit tyrosine kinase receptor activity because the increased activity of tyrosine kinase receptors plays a role in the growth of cancer cells [18].

4. CONCLUSION

The phytochemical screening of ethanolic extract of *meistera chinensis* fruit revealed the presence of the following compounds: phenolics, flavonoids, steroids, terpenoids, alkaloids, and saponins. The BSLT test showed that ethanolic extracts of the *Meistera chinensis* fruit were found highly toxic. From this, it can be concluded that the *Meistera chinensis* fruit is very potential to be used as a medicinal plant and can be developed as a natural anticancer product.

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