

Research Article

Total Phenolic and Flavonoid Content, Antioxidant, and Toxicity Test with BSLT of *Meistera chinensis* Fruit Fraction from Southeast Sulawesi

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Abstract

Zingiberaceae have a large number of species and still need research to reveal their chemical content. *Meistera chinensis* is one of the new generations of the Zingiberaceae family, a local and widespread plant in Konawe Regency, Southeast Sulawesi. Empirically, *M. chinensis* is used as a flavor enhancer in food, aches, and increases body immunity. There are no reports of chemical content or its biological activity. In this study, the fruit of *M. chinensis* was tested for phytochemicals, antioxidants using the ABTS test, total phenolic, total flavonoid content, and toxicity test with a lethal test for brine shrimp (BSLT). *Meistera chinensis* was extracted with ethanol and fractionated by ethyl acetate solvent using vacuum liquid chromatography. Phytochemical screening was carried out qualitatively by using the calorimetric method. The fraction toxicity was monitored by BSLT. These fractions for the ABTS method obtained fractions 1-8 (F1-F8) and ascorbic acid were used as controls. The results showed radical scavenging activities fraction of *M. chinensis* fruit was a very strong activity with IC_{50} of 42.7 ± 3.53 mg/L (F8). The total phenolic and flavonoid contents were 30.72 ± 1.07 mgGAE/g and 8.02 ± 0.48 mgQE/g, respectively. The phytochemical evaluation contains terpenoids, saponins, phenolics, steroids, alkaloids, and flavonoids. The BSLT toxicity test was found to be very toxic, with an IC_{50} of 5.2 ± 0.72 mg/L. These findings indicate that the fruit of *M. chinensis* acts as an antioxidant and toxicity agent.

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INTRODUCTION

The evidence from the last several decades strongly supports the development of modern medicine, and there has been an increasing awareness of the importance of medicinal plants. Plants still contribute to health care as prophylaxis and in primary medicine. There are several reasons for using herbs such as easy to obtain, cheaper, safer, efficient, and rarely have side effects¹. Plants that have been known to cure disease empirically are an option to examine the current search for new

therapeutically effective drugs such as anticancer drugs, antimicrobial drugs, and antioxidants^{2,3}. Various food research results suggest that phenolic secondary metabolites have significant antioxidant activity. Phenolic compounds have properties to prevent and treat various diseases, including diabetes, cancer, cardiovascular, and neurodegenerative diseases^{4,5}.

Zingiberaceae is an important plant family reported with high potential biological activity that can treat various diseases⁶. Zingiberaceae has a large plant kingdom

species in the world, including in Indonesia. Several new generations were discovered by *Cinnamomum*, *Meistera*, and *Wurfbainia* in the newly discovered Zingiberaceae family. Therefore, it is essential to determine plants' medicinal potency by intensifying studies on medicinal plants⁷.

Previous research Zingiberaceae species such as *Etilingera elatior* (ginger torch) function as antioxidants⁸, anticancer⁹, antibacterial, antifungal, cytotoxic, tyrosinase inhibitory activity¹⁰, and immunomodulatory activity¹¹. Literature studies suggest that *E. elatior* has pharmacological activities such as anti-tumor, anti-hyperglycemic, anti-inflammatory, and anti-hyperuricemic¹².

Meistera chinensis is one of the species that belong to the Zingiberaceae family. *Meistera chinensis*, which is the local plant of Southeast Sulawesi and found in the Konawe Regency. Empirically, it is used as a flavor enhancer in food, aches, and increases body immunity.

The safety of potential toxic effects in drug research is vital to ensure their use. The low toxicity and clinical effectiveness of natural compounds are among the researchers' aims to obtain plant biological activity¹³. Toxicity testing is the first step in drug safety parameters before it becomes a drug product used in humans. One of the toxicity testing methods is Brine Shrimp Lethality Test (BSLT). This method can identify the toxicity of natural ingredients¹⁴.

The evidence base of researchers has found that the antioxidants in various plants can be used to treat disease and maintain human health¹⁵. In the human body, antioxidant compounds can maintain optimal health. Antioxidants function as anti-carcinogenic, regenerate cells, and anti-mutagenic¹⁶. Research on plants and fruits shows strong antioxidants activity such as flavonoids, phenolics, and tannins compounds are important bioactive which are efficacious for protection from disease. Consumption of natural antioxidants has been

reported to reduce morbidity and mortality from degenerative diseases^{17,18}.

Currently, healthcare costs increase with disease progression¹⁹. 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, unique properties for patient protection and safety²⁰. Therefore, this study aims to investigate phytochemical screening, total flavonoid and phenolic content, antioxidant, and *in vitro* toxicity activity of *M. chinensis* fruit extract.

MATERIALS AND METHODS

Materials

Meistera chinensis fruit was obtained from Konawe District, Abuki Village, Southeast Sulawesi. The plant collected was authenticated by The Indonesian Institute of Sciences (LIPI), Indonesia. A voucher specimen (601) of the plant was stored at the Herbarium Bogoriense of The Research Center for Biology, Cibinong Science Center. The fresh fruit material (Figure 1), as well as its simplicia (Figure 2), was subsequently cleaned, then dried at 40°C and protected from the sun for four days, and pulverized with a mechanical grinding machine.



Figure 1. Fresh fruit of *M. chinensis*



Figure 2. Simplicia for *M. chinensis* before (left) and after (right) drying

The chemical reagents used include ethanol 95%, *n*-hexane, silica gel 60 GF₂₅₄ (Merck), ethyl acetate, FeCl₃, NaOH, H₂SO₄, chloroform, Na₂CO₃, HCl, Mayer's reagents, formic acid, acetonitrile, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Sigma-Aldrich), potassium persulfate (Merck), ascorbic acid, Trolox (Sigma-Aldrich), *Artemia salina*, potassium dichromate (Merck), dimethyl sulfoxide, anhydrous acetic acid, gallic acid, quercetin, Folin-Ciocalteu's phenol reagent (Sigma), 25% ammonia 2 N, and Mg powder.

Methods

Extraction

About 3,000 g of *M. chinensis* fruit powder dissolved with 95% ethanol in a closed glass container for 3 × 24 hours. The maceration process was carried out for three days and filtered and replaced the solvent. The filtrate was collected and concentrated using a rotary vacuum evaporator at 50°C to obtain a viscous extract of 150 g.

Fractionation by vacuum liquid chromatography

Separation of chemical compounds was carried out by thin-layer chromatography (TLC) to found suitable eluents as references in vacuum liquid chromatography. Columns made using 60 GF₂₅₄ silica gel with a ratio of 1:2. Filter paper as a barrier and silica in the column, then added *n*-hexane to wet the silica. The solvents *n*-hexane and ethyl acetate were varied in degree of polarity based

on the volume ratio (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 v/v). The vacuum pump was started, and the solvent system was slowly poured into the column. This process was repeated for all eluent comparisons.

Qualitative phytochemicals screening

Qualitative phytochemical was carried by standard methods to determine the presence of secondary metabolites compounds of *M. chinensis* fruit, including alkaloids, terpenoids, flavonoids, phenols and tannins, steroids, and saponins^{21,22}.

Alkaloids: About 0.5 g of viscous extract was dissolved with 2 mL of 1% HCl, heated slowly, and filtered. Mayer's Reagent is added to the mixture. The presence of red sediment indicates alkaloids.

Flavonoids: The viscous extract was dissolved with 2 mL of 2% NaOH solution. Flavonoids were indicated by the presence of a viscous yellow color turned colorless with some dilute acids.

Phenols and tannins: The viscous extract was dissolved in 2 mL of 2% FeCl₃ solution. Phenols and tannins were indicated by greenish-blue or black color.

Saponins: The 0.5 g of viscous extract was shaken with 2 mL of distilled water then shaken vigorously. Saponins were indicated by the presence of foam formation.

Steroids: The viscous extract was dissolved with 2 mL concentrated H₂SO₄ and chloroform. Steroids were indicated by the formation of red color in the chloroform layer.

Terpenoids: The thick extract was added with 2 mL of chloroform and dryness. About 2 mL of H₂SO₄ were added and heated until 2 minutes. A grayish color indicates terpenoids.

Determination of total phenolic contents

As much as 10 mg of the extract was dissolved in 10 mL of ethanol. Each pipette was 1 mL of extract solution; then the sample was added with 0.4 mL of Folin-Ciocalteu's reagent, shaken, and left for 4-8 minutes, added with 4

mL 7% Na₂CO₃ solution, and shaken until homogeneous. Distilled water was added to 10 mL and let stand for 2 hours at room temperature. The absorption was measured at a maximum absorption at 765 nm. The total phenolic content was indicated as mg gallic acid equivalent per 1 g of extract weight (mgGAE/g). The sample measurements were replicated three times^{23,24}.

Determination of total flavonoid contents

Total flavonoid contents were measured using the colorimetric method with quercetin as standard. About 10 mg of the extract was dissolved in 10 mL of ethanol. As much as 1 mL of the solution was taken and added with 3 mL of methanol, 0.2 mL of 10% AlCl₃, 0.2 mL of potassium acetate, and distilled water up to 10 mL. The solution was stored for 30 minutes in a dark place at room temperature. The absorbance was measured on UV-Vis spectrophotometry expressed at 420 nm. The total flavonoid content was as mg equivalent of quercetin per 1 g of extract weight (mgQE/g). The sample measurements were replicated three times^{23,24}.

ABTS's radical scavenging activity

Briefly, 2.45 mM K₂S₂O₈ solution was dissolved with 7 mM ABTS solution and stored for 24 hours in the darkroom to produce a colored solution containing the ABTS radical cation. Before being used for testing, the ABTS radical cation was dissolved with 50% methanol, where the initial absorbance was 0.7±0.02 at 745 nm and a temperature of 30°C. Antioxidant activity was determined by 300 µL of 3 mL of ABTS standard solution in a microcomputer. Absorption reduction is carried out exactly 1-6 minutes after mixing. The percentage of inhibition was determined by the formula²⁴:

$$\text{Scavenging effect (\%)} = \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} \times 100$$

Toxicological Evaluation Brine Shrimp Lethality Assay

The BSLT method was used to know plant extracts' toxicity test²⁵. The extract was put in a vial containing 5

mL of seawater and 10 *Artemia salina* leach larvae hatched for 48 hours. The concentrations used in BSLT were 10; 100; 250; 500; 750 µg/mL, and negative control. The percentage of dead shrimp observed after 24 hours and was calculated. Each concentration had three replications. The toxicity test was determined according to the number of dead larvae. The toxicity test was assessed by determining the LC₅₀ score^{14,26}.

$$\% \text{ mortality} = \frac{\text{Total larvae mortality}}{\text{Total larvae}} \times 100$$

The LC₅₀ score is defined as the concentration of a compound causing 50% mortality of shrimp larvae. Data were analyzed by probit in linear regression $y = a + bx$. The level of toxicity of a compound was classified according to Meyer *et al.*²⁵. It was very toxic when the LC₅₀ of ≤30 µg/mL, moderate toxic with LC₅₀ of >30–1000 µg/mL, and low toxic with LC₅₀ of >1000 µg/mL.

Statistical analysis

Total flavonoids and phenolic content were performed by UV-Vis spectrophotometry. Each sample analyzed was replicated three times and was presented as mean (± SD) at least three independent experiments using SPSS version 20.0. Statistical analysis was with ANOVA with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction

About 3,000 g of plant sample extracted with 22.5 L of 95% ethanol and the vacuous extract obtained was 150 g, and the yield value was 5% — maceration process for 3 x 24 hours. The ethanol extract was then dissolved using ethyl acetate solvent and separated between the soluble and insoluble fractions.

Fractionation by vacuum liquid chromatography

The initial fraction with TLC aims to found a solvent system to separate the compound, so its pattern can be

seen. Each fractionation process was analyzed with TLC and determined by UV light 254 and 366 nm, then sprayed with CeSO_4 ²⁷. Vacuum liquid chromatography separation of the ethyl acetate soluble extract resulted in eight fractions analyzed by TLC using hexane : ethyl acetate (8:2) as eluent. The TLC results show the following chromatograms (Figure 3).

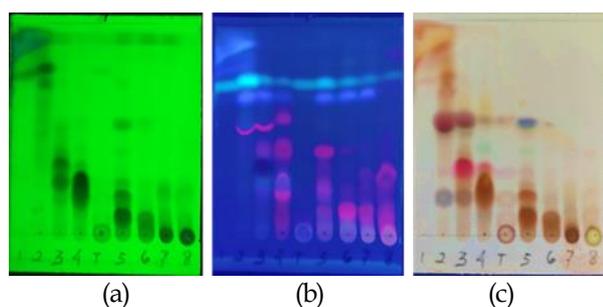


Figure 3. Chromatogram of fractions *M. chinensis* fruit extract with UV light 254 nm (a), 366 nm (b), and cerium sulfate (CeSO_4) (c)

The chromatogram shows differences in compounds from each fraction as indicated by the difference in the Rf value on the appearance of the spots formed (Figure 3). Visualization of detection of separation results was essential in TLC analysis. The UV detection involves using a UV active compound (indicator) put into a stationary phase. Shortwave UV light (254 nm) with an indicator (manganese-activated zinc silicate) would produce a light green color, while the compounds would form dark spots. Longwave UV light (366 nm) with an indicator would produce a purple color. Compounds that absorb 366 nm of UV light would appear as different spots, depending on the compound type. Compounds that do not absorb UV light at 254 nm or 366 nm were spray detected using the oxidizing reagent CeSO_4 in H_2SO_4 to form black spots²⁸. The Rf value of the spot appearance of each fraction was presented in the Table I. The solvent system's determination was carried out using a combination of solvents with low polarity differences to have been mixed. The solvent mixture of *n*-hexane and ethyl acetate was a universal eluent system

often recommended in chromatography because it was easy to evaporate and adjusts polarity (Table I). *n*-hexane and ethyl acetate solvents with different degrees of polarity (9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9 v/v) exhibited excellent separation and were suitable for use as a solvent system in separations using column chromatography.

Table I. The Rf value of *M. chinensis* fruit fraction

No	Fraction	Rf
1	Fraction 1 (F1)	0.96; 0.90; 0.79
2	Fraction 2 (F2)	0.96; 0.90; 0.79; 0.74; 0.65; 0.53
3	Fraction 3 (F3)	0.53; 0.40; 0.34; 0.25
4	Fraction 4 (F4)	0.40; 0.34; 0.25; 0.20
5	Fraction 5 (F5)	0.00; 0.25; 0.20; 0.10
6	Fraction 6 (F6)	0.00; 0.10; 0.05
7	Fraction 7 (F7)	0.05; 0.00
8	Fraction 8 (F8)	0.00

Qualitative phytochemicals screening

Qualitative phytochemical was performed using colorimetric methods to investigate the *M. chinensis* fruit extract's secondary metabolites, including terpenoids, saponins, phenolics, steroids, alkaloids, and flavonoids²⁹. The result presented in Table II shows the presence of phytochemicals.

Table II. Phytochemical evaluation of the *M. chinensis* fruit extract

Phytochemical	Reagent	Chemical reaction	Result
Terpenoid	H_2SO_4 and heated	Grayish color	+
Saponin	Distilled water	Stable foam	+
Phenolic	FeCl_3 2%	Black coloration	+
Steroid	Liebermann-Burchard reagents	Greenish coloration	+
Alkaloid	Mayer's reagents	Precipitate	+
Flavonoid	NaOH 2%	Yellow color	+

Based on Table II, the phytochemical evaluation showed that *M. chinensis* fruit extract contains phytochemicals including terpenoids, saponins, phenolics, steroids, alkaloids, and flavonoids. Several secondary metabolites, including triterpenoids and flavonoids, were potential antioxidant, antibacterial, and toxicity properties. Plants

contain various chemical compounds such as alkaloids, phenols, steroids, terpenoids, and flavones were responsible for many pharmacological properties³⁰.

Total phenolic contents

Quantitative analysis of *M. chinensis* fruit's total phenolic content was performed using Folin–Ciocalteu's reagent and analyzed by UV-Vis spectrophotometer. Phenolic contents react with the Folin–Ciocalteu's reagent and form complex blue compounds. The standard solution used was gallic acid, a phenolic compound derived from hydroxybenzoic acid²⁴. The total phenolic contents from extract of *M. chinensis* fruit could be seen in **Table III**.

Table III. Total phenolic contents from ethanol extract of *M. chinensis* fruit

Replication	Absorbance	Total phenolic (mgGAE/g)	Rate of total phenolic (mgGAE/g)
1	0.324	29.57	30.72±1.07
2	0.328	31.68	
3	0.331	30.92	

Based on **Table III**, the total phenolic in the ethanol extract of *M. chinensis* fruit was 30.72±1.07 mgGAE/g, that each g of the extract was equivalent to 30.72 mg of gallic acid. This study stated that the formation of complexes by phenolic compounds with carbohydrates and proteins, obtained from the solvent methanol in the extraction process compared to other solvents. Secondary metabolites phenolics or polyphenols were very important for plants because they provide antioxidant activity to chelate redox-active metal ions, lipid-free radical chains, and block conversion hydroperoxides to reactive ones' oxyradicals³¹.

The presence of total phenolic contributes to the antioxidant activity³². The total phenolic in the extract depends on the polarity of the solvent used in the extraction. The high solubility of phenol compounds in polar solvents gives the extract a high concentration³³.

Total flavonoid contents

The *M. chinensis* fruit ethanol extract's total flavonoid content was determined by the complex formation between aluminum chloride with the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom from the flavone and flavonol groups. Quercetin was used as a standard for determining flavonoids' levels because quercetin was a flavonol group with a keto group on the C-4 atom and the hydroxyl group on the C-3 and C-5 atoms³⁴. The results of total phenolic contents could be seen in **Table IV**.

Table IV. Total flavonoid contents from ethanol extract of *M. chinensis* fruit

Replication	Absorbance	Total flavonoid (mgQE/g)	Rate of total flavonoid (mgQE/g)
1	0.200	8.57	8.02±0.48
2	0.179	7.82	
3	0.182	7.68	

The total flavonoid content expressed as mg equivalent per g of dry sample was 8.02±0.48 mgQE/g (**Table IV**). This results in sync with the total phenolic content obtained. Other research had shown that high phenolic levels also contain large amounts of flavonoids³¹.

Plants were a major source of minerals, vitamins, and other essential nutrients. Most of the medicinal plants contain phenolic acids, flavonoids, and other phytochemicals. In the past, the plants was used as medicine to prevent and treat several diseases^{31,35}.

ABTS's radical scavenging activity

ABTS radical cation decolorization was an applied approach to determining radical scavenging activities. It had been studied; the activity of various antioxidant compounds present in plants was determined by the extraction method³¹.

The ethanol extract of *M. chinensis* was fractioned and obtained fraction 1-8 (F1-F8). The value of inhibition of the fraction at the concentration of 0-50 mg/L showed that *M. chinensis* had very strong antioxidant activity (F8)

and ascorbic acid (AA) as a positive control with IC_{50} was 42.70 ± 3.53 and 9.58 ± 0.57 mg/L, respectively¹² (Figure 4).

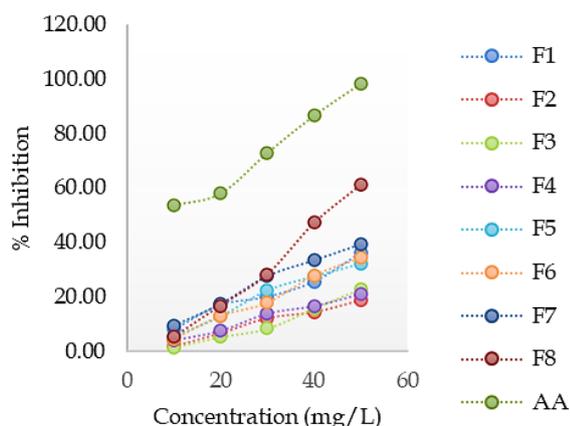


Figure 4. ABTS radical scavenging of *M. chinensis* fruit fraction

Present results showed that the ABTS radical scavenging ability can be ranked as $F8 > F7 > F6 > F5 > F1 > F3 > F2 > F4$ (Figure 4). Based on 50% inhibition concentration, fraction of *M. chinensis* fruit shows very strong antioxidant activity with IC_{50} of <50 mg/L (F8), strong category with IC_{50} of 50-100 mg/L (F1, F5, F6, and F7) and medium category with IC_{50} of 100-150 mg/L (F2, F3, and F4)³⁶.

Plant phenolic compounds had flavonoids that have potent antioxidant activity. Naturally, there were flavonoids in plants that could benefit humans. Studies on flavonoids had demonstrated various antibacterial, antiviral, anticancer, anti-allergic, and anti-inflammatory activities. Flavonoids were very effective at warding off free radicals, which could cure several diseases⁵. Findings in other plant products indicate that flavonoids and phenolic acids were the main contributors to antioxidant agents as the IC_{50} value of free radical activity from various dissolved fractions³¹.

Toxicological Evaluation Brine Shrimp Lethality Assay

The toxicity of the *M. chinensis* fruit extract was measured using the BSLT method. The mortality percentage and LC_{50} of ethyl acetate fraction of *M. chinensis* were shown in Table V.

Table V. The LC_{50} of the *M. chinensis* fruit

Sample	LC_{50} (mg/L)
Ethyl acetate fraction	5.20 ± 0.72
Positive control (potassium dichromate)	2.26 ± 0.60

The LC_{50} was a value that indicates the concentration of a toxic compound that causes the death of organisms up to 50%. The toxicity test for larvae mortality (Table V) showed an LC_{50} value of about 5.20 ± 0.72 mg/L and was included in the highly toxic category (≤ 30 ppm). The LC_{50} of ≤ 30 mg/L is defined as highly toxic; LC_{50} of $\leq 1,000$ mg/L as toxic; and LC_{50} of $>1,000$ mg/L as non-toxic. Toxicity testing was a common method that could be used to discover new types of drugs³⁷.

The BSLT method was based on an extract's toxicity test, which was simple, fast, inexpensive, and accurate³⁸. The LC_{50} toxicity test used a sample of *Artemia salina* leach larvae at extract concentrations of 0, 10, 100, 250, 500, and 750 ppm (Figure 5). The number of larvae that died was calculated using the SPSS probit analysis.

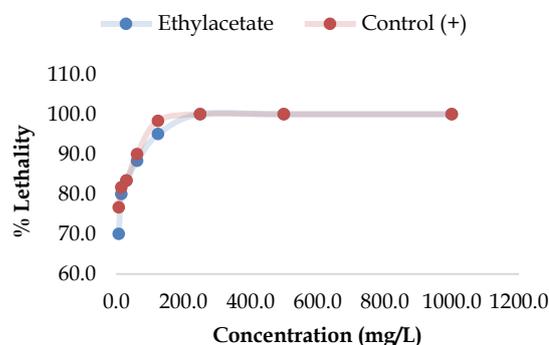


Figure 5. Ratio of toxicity *M. chinensis* fruit fraction and positive control (potassium dichromate) to *Artemia salina* leach larvae

The mortality of shrimp larvae by positive control for potassium dichromate was presented as LC_{50} of 2.26 ± 0.6 mg/L. This result shows that they were not significantly different from the ethyl acetate fraction on shrimp larvae mortality. The highest mortality rate of *Artemia salina* larvae at extract concentrations of 250-750 $\mu\text{g}/\text{mL}$ was 100% (Figure 5). The extract concentration increased with the death of the shrimp larvae. The larva mortality rate

was caused by the concentration of the extract given and the presence of secondary metabolites of the extract (Table II), such as flavonoids, phenolics, and terpenoids. Zingiberaceae was a widespread plant group in Indonesia and used as herbs with antioxidant, cytotoxic, antibacterial, antifungal, hepatoprotective activities^{10,39}.

CONCLUSION

This study's results and published data support this conclusion that antioxidants with ABTS radical scavenging activities fraction of *M. chinensis* fruit show very strong antioxidant activity with an IC₅₀ 42.7±3.53 mg/L (F8). The total phenolic and flavonoid contents were 30.72±1.07 mgGAE/g and 8.02±0.48 mgQE/g, respectively. The BSLT toxicity test was found to be very toxic with an IC₅₀ of 5.2±0.72 mg/L. It shows that mainly the fraction of *M. chinensis* fruit could be the potential source of natural antioxidants and toxicity agents.

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