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. Several reasons for using herbs such as easy to obtain, cheaper, safer, efficient, and rarely have side effects. 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. 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.

Zingiberaceae is an important plant family reported with high potential biological activity that can treat various diseases. 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 Etlingera
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 one of 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, and
degenerative diseases17,18.

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 is 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.
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 x 24 hours. The maceration process was carried out for three days and filtered and replaced the solvent. The filtrate was collected and concentrated by 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 find 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 is started, and the solvent system is slowly poured into the column. This process is 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.

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. Alkaloids indicated by the presence of red sediment.

Flavonoids: The viscous extract was dissolved with 2 mL of 2% NaOH solution. Flavonoids 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 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 indicated by the presence of foam formation.

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

Terpenoids: The thick extract was added with 2 mL of H₂SO₄ and dryness. About 2 mL of H₂SO₄ were added and heated until 2 minutes. Terpenoids indicated by a grayish color.

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\(^{21,24}\).

**Determination of total flavonoid contents**

Total flavonoid contents 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\% AlCl\(_3\), 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 K\(_2\)S\(_2\)O\(_8\) 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 0.74±0.02 at 745 nm and a temperature of 30°C. Antioxidant activity was determined by 300 \(\mu\)L of 3 mL of ABTS standard solution in a microcomputer. Decrease in absorption precisely 1-6 minutes after mixing. The percentage of inhibition is determined by the formula\(^{24}\):

\[
\text{Scavenging effect (\%)} = \left( \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \right) \times 100
\]

**Toxicological Evaluation Brine Shrimp Lethality Assay**

The BSLT method was used to know plant extracts’ toxicity test\(^{25}\). 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 \(\mu\)g/mL, and negative control. The percentage of dead shrimp observed after 24 hours and was calculated. Each concentration has three replications. The toxicity test was determined according to the number of dead larvae. The toxicity test was assessed by determining the LC\(_{50}\) score\(^{14,23}\):

\[
\text{% mortality} = \left( \frac{\text{Total larvae mortality}}{\text{Total larvae}} \right) \times 100
\]

The LC\(_{50}\) 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 is classified according to Meyer et al.\(^{25}\). Very toxic when the LC\(_{50}\) of \(\leq 30 \mu\)g/mL, moderate toxic with LC\(_{50}\) of \(>30\)–1000 \(\mu\)g/mL, and low toxic with LC\(_{50}\) of \(>1000 \mu\)g/mL.

**Statistical analysis**

Total flavonoids and phenolic content were performed by UV-Vis spectrophotometry. Each sample analyzed was replicated three times and were 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. Then the ethanol extract was dissolved by using ethyl acetate solvent and separated between the soluble and insoluble fractions.

**Fractionation by vacuum liquid chromatography**

The initial fraction with TLC aims to find 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$$^2$. Vacuum liquid chromatography separation of the ethyl acetate soluble extract resulted in 8 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)](image)

The chromatogram shows differences in compounds from each fraction as indicated by the difference in the R$_f$ value on the appearance of the spots formed (Figure 3). Visualization of detection of separation results is 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) will produce a light green color, while the compounds will form dark spots. Longwave UV light (366 nm) with an indicator will produce a purple color. Compounds that absorb 366 nm of UV light will appear as different spots, depending on the compound type. Compounds that do not absorb UV light at 254 nm or 366 nm are spray detected using the oxidizing reagent CeSO$_4$ in H$_2$SO$_4$ to form black spots. The R$_f$ value of the spot appearance of each fraction is presented in the Table I.

<table>
<thead>
<tr>
<th>No</th>
<th>Fraction</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fraction 1 (F1)</td>
<td>0.96; 0.90; 0.79</td>
</tr>
<tr>
<td>2</td>
<td>Fraction 2 (F2)</td>
<td>0.96; 0.90; 0.79; 0.74; 0.65; 0.53</td>
</tr>
<tr>
<td>3</td>
<td>Fraction 3 (F3)</td>
<td>0.53; 0.40; 0.34; 0.25</td>
</tr>
<tr>
<td>4</td>
<td>Fraction 4 (F4)</td>
<td>0.40; 0.34; 0.25; 0.20</td>
</tr>
<tr>
<td>5</td>
<td>Fraction 5 (F5)</td>
<td>0.00; 0.25; 0.20; 0.10</td>
</tr>
<tr>
<td>6</td>
<td>Fraction 6 (F6)</td>
<td>0.00; 0.10; 0.05</td>
</tr>
<tr>
<td>7</td>
<td>Fraction 7 (F7)</td>
<td>0.05; 0.00</td>
</tr>
</tbody>
</table>

The solvent system's determination is carried out using a combination of solvents with low polarity differences so that they can be mixed. The solvent mixture of n-hexane and ethyl acetate is a universal eluent system often recommended in chromatography because it is 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) exhibit excellent separation and are suitable for use as a solvent system in separations using column chromatography.

### Qualitative phytochemicals screening

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

<table>
<thead>
<tr>
<th>Phytocemical</th>
<th>Reagent</th>
<th>Chemical reaction</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoid</td>
<td>H$_2$SO$_4$ and heated</td>
<td>Grayish color</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>Distilled water</td>
<td>Stable foam</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic</td>
<td>FeCl$_3$, 2%</td>
<td>Black coloration</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>Liebermann–Burchard reagents</td>
<td>Greenish coloration</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>Mayer’s reagents</td>
<td>Precipitate</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>NaOH 2%</td>
<td>Yellow color</td>
<td>+</td>
</tr>
</tbody>
</table>

Based on Table II, the phytochemical evaluation showed that M. chinensis fruit extract contains phytochemical including terpenoids, saponins, phenolics, steroids, alkaloids, and flavonoids. Several secondary metabolites, including triterpenoids and flavonoids, are potential antioxidant, antibacterial, and toxicity properties. Plants contain various chemical compounds such as alkaloids,
phenols, steroids, terpenoids, and flavones are responsible for many pharmacological properties.

**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 is gallic acid, a phenolic compound derived from hydroxybenzoic acid. The total phenolic content from extract of *M. chinensis* fruit can be seen in Table III.

<table>
<thead>
<tr>
<th>Replication</th>
<th>Absorbance</th>
<th>Total phenolic (mgGAE/g)</th>
<th>Rate of total phenolic (mgGAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.324</td>
<td>29.57</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.328</td>
<td>31.68</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.331</td>
<td>30.92</td>
<td>30.72±1.07</td>
</tr>
</tbody>
</table>

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 are 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' oxyradicals.

The presence of total phenolic contributes to the antioxidant activity. 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 concentration.

**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 is used as a standard for determining flavonoids' levels because quercetin is a flavonoid group with a keto group on the C-4 atom and the hydroxyl group on the C-3 and C-5 atoms. The results of total phenolic contents can be seen in Table IV.

<table>
<thead>
<tr>
<th>Replication</th>
<th>Absorbance</th>
<th>Total flavonoid (mgQE/g)</th>
<th>Rate of total flavonoid (mgQE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.200</td>
<td>8.57</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.179</td>
<td>7.82</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.182</td>
<td>7.68</td>
<td>8.02±0.48</td>
</tr>
</tbody>
</table>

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 has shown that high phenolic levels also contain large amounts of flavonoids.

Plants are 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 use of plants is used as medicine to prevent and treat several diseases.

**ABTS’s radical scavenging activity**

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

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 IC₅₀ was 42.70±3.53 and 9.58±0.57 mg /L, respectively (Figure 4).
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 have flavonoids that have potent antioxidant activity. Naturally, there are flavonoids in plants that can benefit humans. Studies on flavonoids have demonstrated various antibacterial, antiviral, anticancer, anti-allergic, and anti-inflammatory activities. Flavonoids are very effective at warding off free radicals, which can cure several diseases⁵. Findings in other plant products indicate that flavonoids and phenolic acids are 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* are shown in Table V.

**Table V.** The LC$_{50}$ of the *M. chinensis* fruit

<table>
<thead>
<tr>
<th>Sample</th>
<th>LC$_{50}$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate fraction</td>
<td>5.20±0.72</td>
</tr>
<tr>
<td>Positive control (potassium dichromate)</td>
<td>2.26±0.60</td>
</tr>
</tbody>
</table>

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

The BSLT method is based on an extract's toxicity test, which is 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.

The mortality of shrimp larvae by positive control for potassium dichromate was presented as LC$_{50}$ of 2.26±0.60 mg/L. This result shows that they are 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 μg/mL is 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 is a widespread plant group in Indonesia.
and used as herbs with antioxidant, cytotoxic, antibacterial, antifungal, hepatoprotective activities\(^{30,39}\).

**CONCLUSION**

This study's results and published data support this conclusion that antioxidants with ABTS radical scavenging activities fraction of \textit{M. chinensis} fruit show very strong antioxidant activity with IC\textsubscript{50} 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 IC\textsubscript{50} of 5.2±0.72 mg/L. It shows that mainly the fraction of \textit{M. chinensis} fruit can be the potential source of natural antioxidants and toxicity agents.

**ACKNOWLEDGMENT**

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