In vitro antioxidant assay and qualitative phytochemical estimation of Meistera chinensis from Southeast Sulawesi

MUSDALIPAH1, KARMIK1, S A TEE1, A W M YODHA2, I SAHIDIN3 and A FRISTIOHADY3*

1 Pharmacy Study Program, Politeknik Bina Husada, Kendari, Indonesia
2 Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Halu Oleo, Kendari, Indonesia
3 Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Halu Oleo, Kendari, Indonesia

*Email: adryanfristiohady@uho.ac.id

Abstract. Meistera chinensis is one of the species that belong to the Zingiberaceae family. Many antioxidant properties have been reported from this family. Thus, we aim to investigate the antioxidant activity of Meistera chinensis and its secondary metabolites. The ethanol extract of M. chinensis was fractioned and obtained Ethyl acetate Fraction (FEA), Precipitate Fraction (FEN), and fraction 1-8 (F1-F8). The fractions were assayed with DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis-[3-ethylbenzothiazoline sulphonate]) for their scavenging activity in vitro. Ascorbic acid and Trolox were used as control. The phytochemical screening was qualitatively conducted with a colorimetric method. The IC50 value of FEA, FEN, F1-F8, AA, and TX according to DPPH were 35.61±2.22; 47.62±2.93; 90.05±2.27; 105.34±0.83; 97.72±1.16; 112.59±1.22; 72.81±2.36; 66.67±2.55; 59.47±2.23; 40.93±2.68; 8.84±0.69; and 11.45±0.87 mg/L, respectively. Similiar with ABTS method that exhibited IC50 were 37.27±3.43; 52.10±3.29; 94.19±4.23; 110.40±3.01; 102.80±2.66; 115.71±3.14; 73.60±3.20; 61.98±3.94; 42.70±3.53; 9.58±0.57; and 12.55±4.02, respectively. The secondary metabolites in Meistera chinensis were phenolic compounds, flavonoids, steroids, terpenoids, alkaloids, and saponins. In conclusion, we confirmed that Meistera chinensis has antioxidant capacity for scavenging the free radicals, and the metabolites in M. chinensis might play a vital role in its activity.

1. Introduction
Free radical is any molecular species, such reactive oxygen species (ROS) and reactive nitrogen species (RNS), which unstable and highly reactive, thereby damaging biological molecules, including DNA, proteins, carbohydrates, and lipids. These free radicals are derived from normal metabolic processes or exogenous sources, such as cigarette smoking, air pollutants, and X-ray exposure. It leads to stress oxidative, resulting from imbalance free radical production and antioxidant defense [1].

An antioxidant is needed to prevent stress oxidative. Antioxidants are defined as compounds that delay or inhibit the oxidation. Oxidation is a chemical reaction that produces free radicals. Antioxidant defenses the body from free radicals, thus prevent the biological molecules damages [2]. Antioxidants naturally produced in the body, including superoxide dismutase (SOD), catalase, glutathione, glutathione reductase, glutathione peroxidases, and glutathione S-transferases. However, the body needs exogenous antioxidant of the endogenous antioxidant cannot compensate the free radicals [1].
Plants are a source of antioxidants. Many plants provide antioxidant capacity, such as plants belonging to the Zingiberae family. Zingiber officinale, Curcuma longa, and Etingerla elatior provide antioxidant capacity due to phenolics and flavonoid compounds in them [3,4]. Meistera chinensis is one of the plants that belongs to the Zingiberae family. The population of M. chinensis is widely and mainly found in Southeast Sulawesi. It traditionally has been used as spices and immunomodulator. It was suspected of providing similar antioxidant capacity with plants from the same class. E. elatior fruit reported has antioxidant capacity [5]. Thus, this study aims to examine the antioxidant capacity of M. chinensis fruit according to DPPH and ABTS assay and its phytochemical components.

2. Material and methods
2.1. Plant collection and determination
The Meistera chinensis fruit was collected from Abuki Village, Konawe Regency, Southeast Sulawesi. Research Center For Biology determined the sample, Indonesian Institue of Science (No. 601/IPH.1.01/II.07/VI/2020).

2.2. Plant extraction and fractionation
Dried simplicia of M. chinensis fruit (3,000 g) was macerated in 96% ethanol (40 L) for 3x24 hrs. The filtrate obtained was concentrated using a rotary evaporator and yielded 150 g of concentrated extract (5%). The concentrated extract was continued by dissolving with ethyl acetate, and it formed 2 layers: ethyl acetate fraction/ FEA (37 g) and precipitate fraction/ FEN (113 g). The ethyl acetate soluble fraction was fraction with vacuum liquid chromatography (VLC) (silica gel 60 254; n-hexane: ethyl acetate (7:3), v/v) and obtained 15 fractions. The fraction with similar profile was combined and obtained 8 main fractions, which were F1 (2.43 g), F2 (2.7 g), F3 (1.35 g), F4 (1.77 g), F5 (2.12 g), F6 (1.62 g), F7 (6.94 g), and F8 (15.56 g).

2.3. Phytochemical screening
Phytochemical screening of M. chinensis fruit was conducted qualitatively by colorimetric methods [6]:

2.3.1. Phenols and tannin. The crude extract (2 g) was mixed with a 2% solution of FeCl₃ (2 mL). The discoloration indicated the presence of phenols and tannins into a blue-green or black.

2.3.2. Flavonoid. The crude extract (2 g) was mixed with a 2% solution of NaOH (2 mL) and added with a few drops of diluted acid. The presence of flavonoids was indicated by an intense yellow colour formed.

2.3.3. Steroid. The crude extract (2 g) was mixed with chloroform (2 mL), and concentrated H₂SO₄ was added sidewise. The presence of steroids was indicated by the A red colour formed in the lower chloroform layer.

2.3.4. Terpenoids. The crude extract (2 g) was dissolved in chloroform (2 mL) and evaporated to dryness. It was continued by adding 2ml of concentrated H₂SO₄ and heated for about 2 minutes. The presence of terpenoids was indicated by a greyish colour formed.

2.3.5. Alkaloids. 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. Saponins. The crude extract (2 g) was mixed with distilled water (5 mL) in a test tube and shaken vigorously. The presence of saponins was indicated by stable foam formed.

2.4. Antioxidant assay
The antioxidant test in this study was conducted using the DPPH free radical scavenger activity test method based on the Blois (1958) method and the modified ABTS based on the modified Re et al. (1999) method [7,8].

2.4.1. DPPH method. DPPH free radical inhibition measurements were conducted by preparing DPPH in a concentration of 0.1 mM in methanol and extracts in 10, 20, 30, 40, and 50 mg/L. The controls used were ascorbic acid and Trolox. A total of 1 mL of 0.1 mM DPPH solution was mixed with each extract solution (2 mL) and control (2 mL). The mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was measured against the blank at a wavelength of 517 nm using a spectrophotometer (Jenway). Lower absorbance of the mixture, indicating a higher DPPH free radical scavenger activity. Measurements were made in triplicates. The percentage of radical scavenger activity of each extract on the DPPH radical was calculated as % DPPH inhibition (I%) using the following equation:

\[ I\% = \left( \frac{A_o - A_s}{A_o} \right) \times 100 \]  

Ao is the absorbance of the control, and As is the absorbance of the extract solution tested.

2.4.2. ABTS method. The measurement of ABTS free radical inhibition was conducted by making ABTS+ stock solution by reacting 7 mM ABTS (Sigma Aldrich) solution with an equal amount of 2.45 mM potassium persulphate (Merck) solution, then left in the dark at room temperature for 12-16 hours prior used. The ABTS+ working solution was obtained by diluting the stock solution in methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Sample solutions were prepared in concentrations of 10, 20, 30, 40, and 50 mg / mL. Next, 2 mL of the ABTS+ solution was mixed with 1 mL of each extract and incubated in the dark at room temperature for 10 minutes. Controls were made using ascorbic acid and Trolox (Sigma-Aldrich) by mixing control of 2 mL ABTS+ solution with 1 mL of control. Samples were prepared and measured in triplicates. The percentage of inhibitory activity of each extract on the ABTS radical is calculated as% inhibition of ABTS (I%) using the following equation:

\[ I\% = \left( \frac{A_o - A_s}{A_o} \right) \times 100 \]  

Ao is the absorbance of the control, and As is the absorbance of the extract solution tested.

2.4.3. Determination of the IC50 value
Each sample's IC50 is calculated based on the percentage of inhibition to radicals of each concentration of the sample solution. After obtaining the percentage of inhibition (y) of each concentration (x), the points (x and y) are plotted on the coordinate plane, then the line equation \( y = ax + b \) is determined by calculating by linear regression where a and b are constants, x is the sample concentration (mg / L), and y is the percentage of inhibition (%). Antioxidant activity is expressed by Inhibition Concentration 50 (IC50), namely the concentration of the sample (x), which can reduce 50% of radicals (y = 50).
3. Results and discussion

3.1. Qualitative phytochemical screening of extracts ethanol

Phytochemical screening is preliminary step in determining of the constituent in studied plant [9]. Phytochemical screening is a preliminary step in determining the constituent in the studied plant [9]. According to phytochemical screening conducted qualitatively, ethanol extract of M. chinensis fruit contains phenolics, flavonoids, steroids, terpenoids, alkaloids, and saponins. The secondary metabolites are showing in Table 1.

| Table 1. Phytochemical screening of Meistera chinensis ethanol extract |
|---------------------------------------------------------------|
| **Meistera chinensis fruit extract** |
| Phenolic     | + |
| Flavonoid    | + |
| Steroid      | + |
| Terpenoid    | + |
| Alkaloid     | + |
| Saponin      | + |

* + = detected

3.2. Antioxidant assay

The antioxidant is substances that can delay or inhibit any oxidant substrates at low concentration. Antioxidant acts as free radical scavengers due to the presence of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that damage cells. The antioxidant prevents free radicals by scavenging them and promote their decomposition [10,11].

According to antioxidant capacity assay, all fractions used were having antioxidant capacity. The IC$_{50}$ value exhibited parallelism with antioxidant capacity; the lowest IC$_{50}$ indicates the higher antioxidant capacity [12]. The ethyl acetate soluble fraction had the lowest IC$_{50}$ value, with 35.61±2.22 mg/mL for DPPH and 37.27±3.43 mg/mL for ABTS. Followed by F8 with CI$_{50}$ value 40.93±2.68 mg/mL for DPPH and 42.70±3.53 mg/mL for ABTS (Table 2). They were considered good for antioxidant capacity, which had the lowest IC$_{50}$ value. The content of phenolics and flavonoids might be playing a vital role in providing antioxidant capacity.

Phenolic compounds are scavenging free radicals by donating hydrogen atoms, electrons, and chelating metal cations. Flavonoids are also showing similar potency as an antioxidant by stabilizing the reactive oxygen species (ROS) with high reactivity of hydroxyl group in flavonoids, the radical is inactive, resulting in more stable and less reactive radical. Flavonoids belong to the phenolic compound [13-15].

| Table 2. Antioxidant assay of Meisteria chinensis fruit extract |
### Table

| No | Sample                     | IC<sub>50</sub> (mg/mL) | DPPH   | ABTS   |
|----|----------------------------|-------------------------|--------|--------|
|    |                            |                         |        |        |
| 1  | Ethyl acetate fraction (FEA) | 35.61±2.22              | 37.27±3.43 |
| 2  | precipitate fraction (FEN)  | 47.62±2.93              | 52.10±3.29 |
| 3  | F7                         | 90.05±2.27              | 94.19±4.23 |
| 4  | F2                         | 105.34±0.83             | 110.40±3.01 |
| 5  | F3                         | 97.72±1.16              | 102.80±2.66 |
| 6  | F4                         | 112.59±1.22             | 115.71±3.14 |
| 7  | F5                         | 72.81±2.36              | 73.60±3.42 |
| 8  | F6                         | 66.67±2.55              | 70.60±3.20 |
| 9  | F7                         | 59.47±2.23              | 61.98±3.94 |
| 10 | F8                         | 40.93±2.68              | 42.70±3.53 |
| 11 | Ascorbic acid              | 8.84±0.69               | 9.58±0.57 |
| 12 | Trolox                     | 11.45±0.87              | 12.5±4.02 |

### 4. Conclusion

*Meistera chinensis* fruit fraction, which was ethyl acetate soluble fraction were providing antioxidant capacity. The secondary metabolite in the fruit might play a role in providing the free radical scavenger activity.

### Acknowledgement

Research was funded by The Ministry of Research and Technology/National Research and Innovation Agency Republic of Indonesia with scheme grant Penelitian Kerjasama Perguruan Tinggi (PKPT) No: 231/SP2H/LT/DRPM/2019

### References

[1] Lobo V, Patil A, Phatak A and Chandra N 2010 *Pharmacogn Rev.* **4**(8) 118–26
[2] Salehi B, Martorell M, Arbiser JL, Sureda A, Martins N, Maurya PK, Sharifi-Rad M, Kumar P, Sharifi-Rad J 2018 *Biomolecules* **8**(4) 1–11
[3] Wahyuni, Grashellaa SHL, Fitriahea WOI, Malaka MH, Fristiohady A, Imran and Sahidin 2019 *Curr Res Biosci Biotechnol* **1**(1) 13–6.
[4] Danciu C, Vlaia L, Fetea F, Hancianu M, Coricovac DE, Ciurlea SA, Ţoica CM, Marincu I, Vlaia V and Dehelean CA, Trandafirescu C 2015 *Biol Res.* **48**(1) 1–9
[5] Jabbar A, Wahyuni, Malaka M H and Apriliani 2019 *Galenika Journal of Pharmacy* **5**(2) 189-97
[6] Yadav R and Agarwala M 2011 *J Phytol* **3**(12) 10–4.
[7] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C 1999 *Free Radic Biol Med* **26**(9–10) 1231–7
[8] Blois MS 1958 *Nature* **181** 1199–200
[9] Susanti NMP, Budiman INA and Warditiani NK 2014 *J Farm Udayana* **3**(1) 83–6
[10] Young JS and Woodside J V 2001 *J Clin Pathol* **54**(3) 176–86
[11] Pham-Huy LA, He H and Pham-Huy C 2008 *Int J Biomed Sci* **4**(2) 89–96
[12] Li X, Wu X and Huang L 2009 *Molecules* **14**(12) 5349–61
[13] Afanas’ev IB, Dorozhko AI, Brodskii A V, Kostyuk VA and Potapovitch AI 1989 *Biochem Pharmacol* **38**(11) 1763–9

[14] Prakash D, Suri S, Upadhyay G and Singh BN 2007 *Int J Food Sci Nutr* **58**(1) 18–28

[15] Panche AN, Diwan AD and Chandra SR 2016 *J Nutr Sci* **5**(e47) 1–15