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Article

Antioxidant activity of *Momordica charantia* extracts

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ABSTRACT

Momordica charantia is one of the herbaceous plants which widely distributed at tropical and subtropical regions. *M. charantia*, as known as pare, has been used as Indonesian traditional medicine for the treatment of bitter stomachic, laxative, and anthelmintic. This study aims to investigate the antioxidant activity of *M. charantia* leaves extracts (the *n*-hexane, dichloromethane, ethyl acetate, and methanol extracts) by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The result showed that the ethyl acetate extract has the highest DPPH scavenging activity with IC₅₀ value of 233.39 µg/mL. Therefore, this study concluded that ethyl acetate extract of *M. charantia* leaves is recommended as an antioxidant agent.

I. INTRODUCTION

Momordica charantia L. is one of the herbaceous plant in the genus *Momordica* of *Cucurbitaceae* family, which distributed in tropical and subtropical regions (Liu et al., 2010). Some Indonesia regions called it as "pare", and in Bali, *M. charantia* known as "paye". Pare has a bitter characteristic. The fruits, leaves, roots of *M. charantia* have been used as a number of diseases therapies in Ayurveda, such as bitter stomachic, laxative, and anthelmintic. The leaves of *M. charantia* have been used for herbal drinks which is called as "loloh" in Bali. Balinese people used "loloh paye" which is contained *M. charantia*

leaves for heartburn and diabetes (Tengah 1995).

In Asian countries, this plant is widely used extensively in folk medicine as remedy for diabetes (Krishnaiah et al., 2011; Chu et al., 2000; Newman et al., 2000). Many phytochemical researches concentrate on the fruit, stem, and seed of *M. charantia* to obtain active compound. The previous research has been reported that the plant contains triterpenoid (Cao et al., 2011; Fatope et al., 1990). The crude extract of *M. charantia* showed antioxidant activity (Andarwulan et al., 2010; Kubola and Siriamornpun 2008), antidiabetic (Peng et al., 2012; Mahomoodally et al., 2007; Matsuur et al., 2002), and antitumor (Singh et al., 1998).

The role of free radical and active oxygen in the pathogenesis of human diseases has been studied. The electron acceptors, such as molecular oxygen, react rapidly with free radical to become reactive oxygen species (ROS). The interesting attention has been focused on the using of antioxidant and trying to develop natural product antioxidant compounds. The antioxidant compounds obtained from plant is carotenoids, phenolic acid and vitamin C. Our previous studies on natural product, plant of *Chromolaena odorata* (Putri et al., 2019), *Ananas comosus* (Putri et al., 2018), *Syzygium polyanthum* (Hidayati et al., 2017), *Moringa oleifera* (Fitriana et al., 2016), and *Cajanus cajan* (Ersam et al., 2016) to possess antioxidant activity.

The previous investigation had shown that water extract of leave and fruit of *M. charantia* exhibited a high value of antioxidant activity with IC_{50} 9.72 ± 0.25 mg/mL in DPPH method (Kubola and Siriamornpun 2008). The results showed that *M. charantia* have rich phenolic compounds that can decrease free radical effects. In addition, the previous studies also have reported that *M. charantia* showed bioactivity as an antidiabetic (Joseph et al., 2013), antibacterial (Khan et al., 1998) and anticancer (Alshehri 2016). Furthermore, some triterpenoid compounds have been isolated such as momordicine I, momordicine II (Bing et al., 2008), 3β -hydroxymultiflora-8-en-17-oic acid, cucurbit-1(10)-5,22,24-tetraen-3 α -ol, $5\beta,19\beta$ -epoxycucurbit-6,22,24-trien-3 α -ol (Liu et al., 2010), momordicoside Q, momordicoside R, momordicoside S, momordicoside T, momorcharaside B, and caravyloside XI (Cao et al., 2011).

In this study, the antioxidant activity of *M. charantia* leaves has been determined. Concerning the special chemical properties of formed free radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay has been widely used to find out the antioxidant ability of food

commodities. In the present work, the antioxidant of various extracts of *M. charantia* will be determined by using DPPH assay.

II. METHODS

Materials

M. charantia leaves were collected from Bali, Indonesia. *M. charantia* leaves was dried in room temperature for extraction. The analytical grade of organic solvent had been used such as methanol, ethyl acetate, dichloromethane, and *n*-hexane. Antioxidant assay used DPPH radical and gallic acid as a standard.

Extraction

The leaves were dried in room temperature and ground into simplicial powder. Twenty gram of *M. charantia* leaves were extracted with 250 mL methanol, ethyl acetate, dichloromethane and *n*-hexane, respectively. The liquid extracts were filtered than evaporated to get various crude extracts.

DPPH Assay

A DPPH assay was evaluated based on our previous method (Putri and Fatmawati 2019). The sample solution was prepared by dissolving 10 mg of the test sample dissolved in 1 mL of methanol. 33 μ L of sample solution was added with 1 mL of DPPH solution (319 ppm). The mixture was vortexed with a mixer and incubated at 37°C for 20 minutes. Antioxidant activity can be observed by changing the color of the solution from purple to yellow and its absorbance can be measured at 515 nm by using spectrophotometer UV-Vis. A blank solution was prepared by dissolving 1 mL of DPPH into 33 μ L of methanol. The calculation of inhibition is calculated based on the following equation:

$$\text{Inhibition (\%)} = \frac{A_b - A_s}{A_b} \times 100\%$$

A_b = Blank absorbance

A_s = Sample absorbance

III. RESULT

The antioxidant activity test of the DPPH method was carried out by adding 1 mL of DPPH solution to the extract sample. The solution was incubated at 37°C for 20 minutes. The presence of a reaction between DPPH and antioxidant compounds is indicated by a change in the color of the purple solution to yellow. The color change occurs due to the presence of hydrogen radical donors from antioxidant compounds to DPPH radicals. The absorbance of the solution was measured at a wavelength of 515 nm using a UV-Vis instrument.

The ethyl acetate extract showed the highest percentage of inhibition, which was $65.65 \pm 0.02\%$ at a concentration of 319 $\mu\text{g/mL}$ compared to the other three extracts, including

methanol extract $40.57 \pm 0.06\%$, methylene chloride $39.48 \pm 0.05\%$ and *n*-hexane $25.07 \pm 0.1\%$ (Fig. 1). The IC_{50} determination was carried out on extracts that had an inhibition value above 50%, namely ethyl acetate extract. The IC_{50} value is the minimum sample concentration that can inhibit DPPH radicals by 50%. The IC_{50} value was done by measuring the inhibition above 50% to the inhibition value below 50% with variations in concentration. Next, a curve of the relationship between the concentration and the percentage of inhibition was made. Based on the interpolation curve (Fig. 2) between the concentration and the percentage of inhibition ($y = 0.1886x + 5.983$; $R^2 = 0.966$), the IC_{50} value of the ethyl acetate extract was 233.39 $\mu\text{g/mL}$.

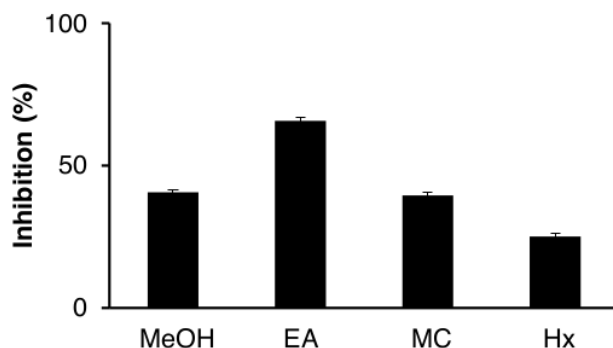


Fig 1. The antioxidant activity of *M. charantia* extracts (MeOH: methanol, EA: ethyl acetate, MC: dichloromethane, Hx: *n*-hexane)

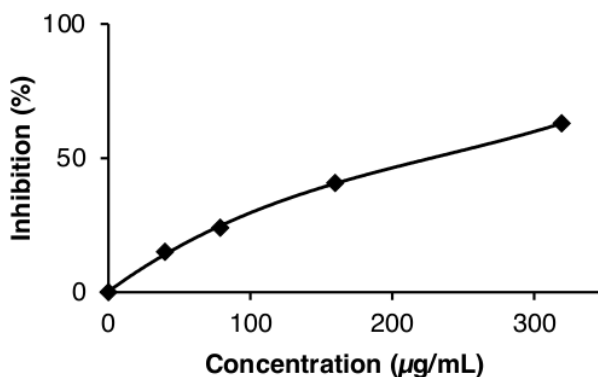


Fig 2. The antioxidant activity of *M. charantia* ethyl acetate extract

IV. DISCUSSION

The number of free radicals in the body that cause degenerative diseases such as diabetes, coronary heart disease, cancer and aging. Increasing free radicals in the body can be released oxidative stress. Oxidative stress is a condition in the body that occurs because of an imbalance between antioxidants and free radicals. Those free radicals can be prevented by antioxidants (Tapan, 2005). Plant diversity in Indonesia is a source of compounds - natural products that can be used as antioxidants. Based on the source, antioxidant compounds are grouped into two types, namely natural antioxidant compounds and synthesis. Synthesis antioxidant compounds have high compatibility, but not necessarily safe for health. Natural antioxidants are obtained by utilizing natural compounds contained in plants (Newman, 2000). Some sources of natural antioxidants contain vitamin C (ascorbic acid), vitamin E (tocopherol), vitamin A (carotenoids), some polyphenols, flavonoids, anthocyanins, and lycopene.

Bali is part of Indonesia's islands that have very strong local wisdom. Bali is a tourist destination that has been known to the world since 1970. Since now many foreign tourists have come to Bali to enjoy wealth, natural beauty and cultural uniqueness. One of the interesting things is its natural beauty. The potential of natural products has been utilized in the form of spas and various castle (home) treatments. Balinese people in their daily lives use plants as a means of healing diseases. The use of plants as a medicinal medium has been inherited traditionally as a tradition of medicine commonly referred to as "Usadha". Usadha is the science of traditional Balinese medicine, whose source of teaching is Lontar. Lontar is a book that is a source of traditional Balinese medicine.

One of the most popular lontar is Lontar Rukmini Tatwa, which uses a system of medicine, medicine and traditional medicine in Bali (Tengah, 1995). The diversity of medicinal plants in Bali is a source of natural ingredients compositions that can be used in alternative medicine specifically in diseases such as cancer, diabetes, coronary and stroke.

There are some methods have been used to investigate antioxidant activity of plants. In the present work, antioxidant activities of *M. charantia* extracts are performed by using DPPH method. The principle of DPPH assay is the radical decolorization through electron transfer which marked with color change from dark purple to become yellow light.

In this present study, the antioxidant activity of *M. charantia* extracts should be affected by the solvent that used for extraction. Every solvent extracted different components based on their solubility. Ethyl acetate is a semi-polar solvent which extracted more components than other solvent. This result contributed for the extract to have the highest antioxidant activity. Hence, the ethyl acetate extract is recommended for chemical constituents isolation process.

V. CONCLUSION

In conclusion, antioxidant activity of *M. charantia* with various extracts (methanol, ethyl acetate, dichloromethane, *n*-hexane extracts) were determined by using DPPH assay. The ethyl acetate extract showed the highest antioxidant activity. Further investigation is needed to clarify the chemical constituents of *M. charantia* which are responsible as an antioxidant agent.

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