DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF *Wedelia trilobata* FLOWER FROM SAMARINDA CITY, INDONESIA

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ABSTRACT

Research on antioxidant activities of aerial parts of seruni (*Wedelia trilobata*) have been conducted and researchers reported that *W. trilobata* flower extracts have antioxidant activities. Some studies were reported that some plants with different location sources show different secondary metabolites and activities levels. This research was designed to investigate antioxidant activity of methanol extract of *W. trilobata* flowers. The flowers were collected from *W. trilobata* plant that wildly grew on land around Samarinda city, East Kalimantan, Indonesia. The dried flowers were extracted using maceration technique with methanol. The extract solution was concentrated using a rotary evaporator at temperature of 40°C. The yielded crude extract was analyzed its antioxidant activity using DPPH radical scavenging method, and its total phenolics contents (TPC) and total flavonoids contents (TFC) were analyzed using Folin Ciocalteu and Aluminium Chloride methods. The result shows that the extract has ability to scavenge DPPH radicals and its activity reached 92.77±0.49% at the extract concentration of 0.050±0.00 mg/ml, and it’s TPC and TFC were 145.83±5.89 mg GAE/g extract and 26.82±0.00 mg QE/g extract. This research found that the methanol extract of *W. trilobata* flower from Samarinda was high in DPPH radical scavenging activity.

Keywords: Antioxidant, flavonoid, phenolic, seruni

INTRODUCTION

*Wedelia trilobata* (L.) Hitch, called seruni in Indonesia, is a vine plant belonging to Asteraceae family (Saleha, et al., 2013; Anonim, 2020). Its stem is rounded, reddish green in color, and grows up to 40 cm long and regularly develop roots at the stem nodes (Balekar, et al., 2012; Balekar, et al., 2014). Leaves are obovate to obovate-lanceolate, bright shiny green, 7–8 cm long, 2-5 cm wide, and borne on short stalks (Balekar, et al., 2012; Balekar, et al., 2014). Flowers are yellow, 4–5 cm in diameter and similar to sunflower, but its size is small (Balekar, et al., 2012; Saleha, et al., 2013).

This plant is usually used as hedge and ornamental plants (Saleha, et al., 2013). This plant has also been used as a traditional herbal medicine for the treatment of abdominal pains, arthritic painful joints, arthritic pain fever, backache, bronchitis, colds, dysmenorrheal, malaria, muscle cramps, rheumatism, stubborn wounds, sores, swellings, even as a fertility enhancer (Ren, et al., 2015; Luyen, et al., 2019). Together with leaves, flowers of the plant were used for amenorrhea and childbirth (Sureshkumar, et al., 2011). Mixture of flowers, stems and leaves are boiled in water
and the extract is used for hepatitis, indigestion due to sluggish liver, white stools, burning in the urine and stopping of urine and for infections (Chethan, et al., 2012). Some extract from the W. trilobata flower have been reported its biological activities. Ethanol extract of W. trilobata flower has antibacterial and anti-inflammatory activities (Govindappa, et al., 2011). Ethanol and methanol extract of the flower of the plant has antioxidant activity to scavenge DPPH radicals (Govindappa, et al., 2011; Chethan, et al., 2012).

Some research on the chemical constituents of the flower of W. trilobata have been also conducted. The flower consists of diterpenoid, sesquiterpenoids and triterpenoid (Balekar, et al., 2014). Phytochemical screening conducted by Husain, et al. (2015) shows that alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, and terpenoids were presented in methanol extract of W. trilobata flower from India. However, research on the secondary metabolites and biological activities of W. trilobata, including its flower, from Samarinda city has not been reported.

Some research were reported that plants from different location sources show different secondary metabolites and biological activities levels. Level of the TFC of Ecummia ulmoides from different growing location in China was different (Dong, et al., 2011). Concentration of polyphenols, as well as antibacterial, anticancer and antioxidant activities of aqueous and ethanol extract of Etilingera elatior flowers from north of Malaysia were higher than that of the E. elatior from south of Malaysia (Ghasemzadeh, et al., 2015). Number of secondary metabolites, as well as the larvacidal activity of acetone and ethyl acetate extracts of Lansium domesticum Corr var Duku leaves and peels from Simpang Agung village was higher than that of the L. domesticum leaves and peels from Rengas Bandung village (Salim, et al., 2016).

Based on the reasons above, this research was conducted to investigate the antioxidant activity of methanol extract of W. trilobata flowers from Samarinda city, East Kalimantan, Indonesia.

**MATERIALS AND METHODS**

**Sample and chemicals**

Flowers of W. trilobata were collected from wild W. trilobata located in Samarinda city, East Kalimantan province, Indonesia in April 2019. The sample was identified by Dr. Syafirizal, M.P., Anatomy and Systematics Plant Lab., Math and Natural Science Faculty of Mulawarman University. The voucher specimen (XIX.Ast.W/1) was deposit at Chemistry Lab., Teacher Training and Education Faculty of Mulawarman University. Methanol used to extract the sample was commercial grade and other chemicals were analytical grade and purchased from Merck KGaA (Germany) and Sigma Aldrich GmbH (Germany).

**Preparation of crude methanol extract of W. trilobata flowers**

The preparation of the sample was using the procedure by Nurlaili, et al. (2019) with few modifications. The W. trilobata flowers were separated from its stalks and petals. They were dried under shade for 7 days. A total of 179.602 g of dried flowers were extracted with 1.5 liters methanol for 24 hours. The extract solution was carried out by filtration. The extraction process was repeated for 3 times. The solution was evaporated using a rotary evaporator (EYELA 1L) at 40°C and then kept in an oven (Memmert type UNB 400) at 40°C for a week to yield crude methanol extract.

**Phytochemical Screening test**

The phytochemical screening test of the crude methanol extract of W. trilobata
flower (CMWF) was conducted using procedure described by Sukemi (2016).

**Determination of total phenolics and flavonoids contents**

Total phenolics content (TPC) was analyzed using Folin Ciocalteu method (Sukemi, et al., 2015). Two milliliter of 0.1 mg/ml CMWF was added with 1.0 ml of 0.2 N Folin Ciocalteu. After 5 minutes, a volume of 0.8 ml of 7.5% sodium carbonate was added into the reaction mixture. The mixture was homogenized and left for 2 hours at room temperature. Then, the absorbance of the reaction mixture was monitored at 760 nm using UV-Vis spectrophotometer (Thermo Spectronic type Genesys 10). The TPC was calculated as milligram gallic acid equivalent (GAE)/g CMWF. Total flavonoids content (TFC) was evaluated using aluminium chloride method (Sukemi, et al., 2015). A volume of 1 ml aluminium chloride (2%) was added into 1 ml of the CMWF (0.1 g/ml) and followed by homogenization. After 1 hour, absorbance of the reaction mixture was measured using UV-Vis spectrophotometer (Thermo Spectronic type Genesys 10) at 420 nm. The TFC was calculated as milligram quercetin equivalent (QE)/g CMWF.

**DPPH radical scavenging activity**

Antioxidant activity of the CMWF was analyzed using DPPH radical scavenging assay describe by Sukemi, et al. (2015) with few modifications. One milliliter of 0.04 mg/ml of DPPH radical methanol solution was added to 1 ml of each variation concentrations of CMWF and followed by homogenization. The mixture was incubated at room temperature and dark condition. After 30 minutes, the absorbance of the reaction mixture was measured using UV-Vis spectrophotometer (Thermo Spectronic type Genesys 10) at 517 nm. A positive control (ascorbic acid) was used. The DPPH radical scavenging activity of the CMWF was calculated as % scavenging activity (SA) follows

\[
SA(\%) = \left[1 - \frac{(A1 - A2)}{A0}\right] \times 100
\]

where A0 is the absorbance of DPPH without the CMWF extract, A1 is the absorbance of DPPH with the presence of the CMWF extract and A2 is absorbance of the CMWF extract without DPPH solution.

**RESULTS AND DISCUSSION**

The CMWF was a dark brown soft solid with percent yield of 18.92% (w/w). Based on the phytochemical tests, the CMWF contained alkaloids, flavonoids, phenolics, tanins, and saponins. Previous research reported that methanol extract of *W. trilobata* flower from Durg District of Chhattisgarh, India, contained alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, and terpenoids (Husain, et al., 2015). The TPC and TFC of the CMWF were 145.83±5.89 mg GAE/g CMWF and 26.82±0.00 mg QE/g CMWF.

Ability of an extract to be a hydrogen donor can be analyzed using DPPH radical scavenging assay. As shown in figure 1, ascorbic acid shows good activity with SA of 93.17±0.00% at the concentration of 0.0003 mg/ml. It is clear that the SA of the CMWF was much lower with the SA of 31.50±3.16% at the concentration of 0.0003 mg/ml. However, the CMWF was high in its activity to scavenge DPPH radicals at the concentration of 0.0006 mg/ml with SA of 57.66±0.49%, and reach 87.26±0.00, 89.85±0.00 and 92.77±0.49% at the concentration of 0.0013, 0.0100 and 0.0500 mg/ml. This result shows that the CMWF is a hydrogen donor agent to stabilize the DPPH radicals.
Previous study conducted by Chethan, et al. (2012) reported that DPPH radical scavenging activity of CMWF from Srirangaptna, India, was 50% at the concentration of 0.06 mg/ml. It shows that the DPPH radicals scavenging activity of the CMWF from Samarinda is about 100 times higher than that of the CMWF from Srirangaptna. The antioxidant activity of the CMWF might be caused by its flavonoids and phenolics contents. Positive relationship between total phenolics content and antioxidant activities from some plant extract has been reported (Ghasemzadeh, et al., 2010; Kefayati, et al., 2017; Shi, et al., 2018). Phenolics and flavonoids function as free radicals’ scavengers (Ghasemzadeh, et al., 2011).

CONCLUSION

This research shows that the methanol extract of W. trilobata flower has the ability to eliminate DPPH radicals with a percentage reaching 92.77±0.49% at the concentration of 0.0500 g/ml. Thus, the methanol extract of W. trilobata flower can be considered as an antioxidant agent.

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