# Antioxidant Activity of *Moringa oleifera* Extracts

**Wiwit Denny Fitriana[1,2], Taslim Ersam[1], Kuniyoshi Shimizu[3], and Sri Fatmawati[1,*]**

*[1]Department of Chemistry, Sepuluh Nopember Institute of Technology (ITS)*
*Jl. Arief Rahman Hakim, Sukolilo, Surabaya 60111, Indonesia*

*[2]Faculty of Mathematics and Natural Sciences, Universitas Pesantren Tinggi Darul Ulum, Jombang 61481, Indonesia*

*[3]Department of Agro-environmental Science, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan*

*Received November 20, 2015; Accepted May 4, 2016*

## ABSTRACT

Moringa oleifera *have been evaluated for its antioxidant activity.* M. oleifera *leaves were extracted with methanol, ethyl acetate, dichloromethane and n-hexane. The antioxidant activity of extracts were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay and an improved 2,2'-azino-bis-[3-ethylbenzothiazoline sulphonate] (ABTS) radical cation decolorization assay in vitro. Trolox was used as standard with $IC_{50}$ 5.89 µg/mL in DPPH assay and 3.06 µg/mL in ABTS assay. The methanol extract showed the highest free radical scavenging activity with $IC_{50}$ value of 49.30 µg/mL in DPPH assay and 11.73 µg/mL in ABTS assay. This study provided that* M. oleifera *leaves possess antioxidant.*

***Keywords***: Moringa oleifera *leaves; antioxidant; DPPH; ABTS*

## ABSTRAK

*Aktivitas antioksidan dari ekstrak daun kelor (*Moringa oleifera*) telah diteliti. Daun kelor diekstrak daunnya dengan metanol, etil asetat, dikolorometana, dan n-heksana. Pengujian aktivitas antioksidan dari masing-masing ekstrak dilakukan dengan metode pengukuran penangkapan radikal oleh 1,1-difenil-2-pikrilhidrazil (DPPH) dan metode penghilangan radikal kation oleh 2,2'-azino-bis-[3-etilbenzotiazolin sulfonat] (ABTS) secara in vitro. Trolox digunakan sebagai kontrol positif dengan nilai $IC_{50}$ 5,89 µg/mL uji DPPH dan 3,06 µg/mL pada uji ABTS. Ekstrak metanol daun kelor menunjukkan nilai aktivitas paling tinggi dengan nilai $IC_{50}$ = 49,30 µg/mL pada uji DPPH dan $IC_{50}$ = 11,73 µg/mL pada uji ABTS. Penelitian ini menjadi bukti ilmiah bahwa daun kelor memiliki aktivitas antioksidan yang tinggi.*

***Kata Kunci***: Moringa oleifera*; antioksidan; daun kelor; DPPH; ABTS*

## INTRODUCTION

Free radical and reactive oxygen species are well known as inducers of cellular and tissue pathogenesis which is causing some diseases like diabetes, cancer, inflammatory and also cardiovascular. Free radical reactions take place in the human body and food systems can causing injury and death [1]. Free radicals are one of the main factors which necessary to cause DNA mutation, which is involve in the initiation stage of carcinogenesis [2]. Reactive Oxygen Species (ROS) are constantly produced in human body by normal metabolic system. An over-production of reactive oxygen can occur the imbalance of defense system. Therefore, investigations of antioxidants are needed which focused on natural compounds from natural sources.

The most widely used synthetic antioxidants in food are butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA). Both of them are very effective as antioxidants but their use in food products is not popular anymore due to their instability and also due to a suspected action as promoters of carcinogenesis. For this reason, there is another interest in the studies of natural healthy (non-toxic) additives as potential antioxidants [3]. The total antioxidant capacity values should include methods applicable to both lipophilic and hydrophilic antioxidants, with regards to similarity and differences of both hydrogen atom transfer and electron transfer mechanism [4]. Some methods have been used to evaluate antioxidant activities of natural compounds by using stable free radical DPPH and ABTS [5].

Most of the antioxidant compounds derived from plant source have wide variety and chemical properties. The antioxidant characteristic is based on its ability to trap free radicals. *Moringa oleifera* (Moringaceae; Indonesian name: kelor) is an ornamental plant native in tropical and subtropical

* Corresponding author. Tel/Fax : +62-31-5943353/5928314
Email address : fatma@chem.its.ac.id

areas, and commonly cultivated in all region of Indonesia as a vegetable for cooking purposes. All parts of this plant had been reported to have variously biological activities such as reducing hyperglycemia [6] anti-inflammatory, anti-diabetic, antimicrobial, anticancer and antioxidant [7]. Kelor Leaves extracts were also found have antioxidant activities with linoleic acid, α-tocopherol and sunflower oil [8]. In Asia and Africa, the leaves is recommended as a supplement because of rich in nutrients for breastfeeding mothers and infant [9]. Nitrile compounds, mustard oil glycosides, benzyl glycosides, phenolic glycosides, flavonoid glycosides, thiocarbamate glycosides and amino acids had been isolated from this plant [7]. The antioxidant activities extract MeOH of *M. oleifera* leaves showed the $IC_{50}$ = 1.60 ± 0.03 mg/mL in DPPH assay and $IC_{50}$ = 1.02 ± 0.06 mg/mL in ABTS assay) [10]. *M. oleifera* leaves contain of natural source of polyphenol that potential to have antioxidant. The purpose of this paper is to evaluate the antioxidant activity of various extract of *M. oleifera* leaves.

## EXPERIMENTAL SECTION

### Materials

Plant materials is *M. oleifera* leaves from Jombang, East Java, Indonesia. Solvent methanol, ethyl acetate, dichloromethane, n-hexane, DMSO and ethanol were purchased from Wako Pure Chemical Industries Japan. The DPPH and ABTS reagent were purchased from Wako (Japan). Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid; Sigma Aldrich) was used as antioxidant standard. All other reagents were of analytical grade.

### Instrumentation

Incubator EYELA SLI-400 used to process incubation of sample. The reaction was monitored by spectrophotometer (UV Jasco V-530, Japan).

### Procedure

#### *Plant and extraction*

The leaves of *M. oleifera* were collected during December to February, 2013 from Jombang, East Java. The leaves of *M. oleifera* dried in room temperature and ground into powder. Twenty grams of leaf powder were extracted with 250 mL of solvent (methanol, *n*-hexane, ethyl acetate, and dichloromethane). The liquid extracts were filtered with filter paper. The filtrates were evaporated to remove the solvent and get four crude extracts.

#### *Antioxidant assay*

**DPPH radical scavenging assay.** The radical scavenging activity of *M. oleifera* extracts against the DPPH radical was determined by the method of Brand Williams with slightly modified by Dudonńe et al. [11-12]. Determination procedures were as follow: 1 mL of $6 \times 10^{-5}$ M DPPH radical solution (prepared daily) was mixed with 33.33 µL of methanolic solutions of *M. oleifera* extracts (maximum dissolved concentration). After 20 min incubation for at 37 °C, absorbance decrease of the mixture was monitored at 515 nm (*As*). During reduction by the antioxidant, the solution colour changed from violet to yellow pale. DPPH radicals have an absorption maximum at 515 nm. Blank samples with 33.33 µL of methanol in the above DPPH radical solution were prepared and measured daily at same wavelength (*Ab*). Trolox was used as positive control. The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula.

$$\text{Inhibition rate}\,(\%) = \left[\frac{\text{Ab - As}}{\text{Ab}}\right] \times 100 \qquad (1)$$

The 50% inhibitory concentration ($IC_{50}$) was expressed as the quantity of the extracts to react with a half of DPPH radicals.

**Radical scavenging by ABTS radical.** ABTS assay was mostly based on the methods described previously [13]. The ABTS method was used based on the ability of antioxidant molecules to quench the long live ABTS·+. The ABTS·+, in which the oxidant, was generated by peroxydisulfate oxidation 2,2' azino - bis (3-ethylbenzothiazoline-6-sulfonic acid). Briefly, the ABTS radical solution was prepared with 5 mL of 7 mM ABTS in ammonium aqueous solution, and then 88 µL of 140 mM potassium persulfate ($K_2S_2O_8$) was added then allowed to stand at room temperature for 12-16 h to yield a dark blue solution. This solution was subsequently diluted with ethanol 99.5% before use which gave an absorbance of 0.7 ± 0.02 at 734 nm as working solution. One milliliter of working solution was mixed with 10 µL of *M. oleifera* extract (maximum dissolved concentration) and shaken well for 10 sec; after 4 min of incubation at 30 °C, the absorbance of the reaction mixture was measured at 734 nm to give *As* values. Trolox was used as positive control. Ethanol 99.5% was used as a blank (absorbance was *Ab*). The antioxidative activity of the *M. oleifera* extracts calculated by determining the decrease in absorbance at different concentrations by using equation 1.

#### *Statistical analysis*

Values of experimental results shown in figures were the mean of at least three determinations (± standard deviation).

## RESULT AND DISCUSSION

Antioxidant from natural source can improve the antioxidant system in body for scavenging free radicals. An interest in antioxidant from natural sources increasing faster than synthetic sources. Phenolic compounds which naturally present in *M. oleifera* plant can reduce the risk of many diseases and its effects which correlated with the antioxidant compounds. Recently, there are some reports about *M. oleifera* leaves which rich in phenolic compounds such as flavonoids, gallic acid, quercetin and kaempferol as antioxidant activity [14]. *M. oleifera* is a one of Indonesian traditional plant that has multipurpose biological activities. *M. oleifera* extracts were evaluated for antioxidant activity by using DPPH and ABTS assays.

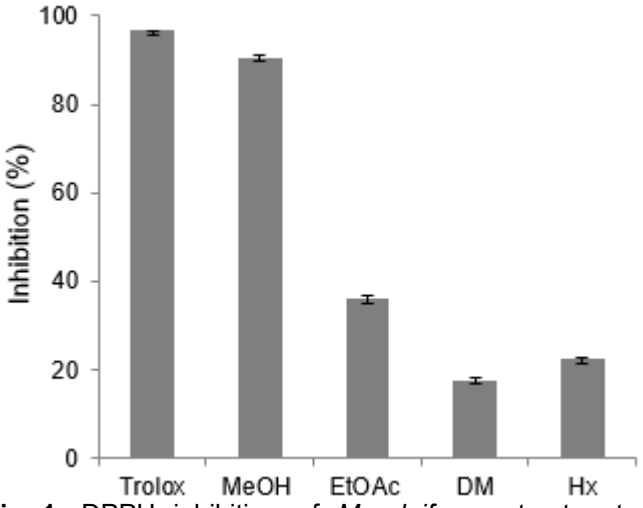


**Fig 1.** DPPH inhibition of *M. oleifera* extracts at a concentration of 319.45 µg/mL; values are mean ± SD of three independent experiments in triplicate at each concentration. MeOH, methanol extract; EtOAc, ethyl acetate extract; DM, dichloromethane extract; Hx, hexane extract

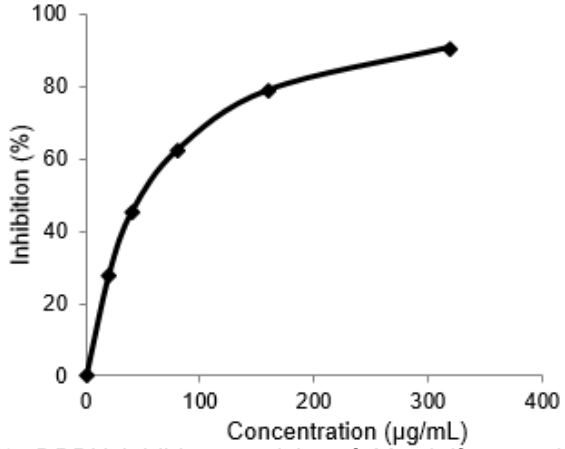


**Fig 2.** DPPH inhibitory activity of *M. oleifera* methanol extract

### DPPH Radical Scavenging Assay

The relatively stable DPPH radical had been used widely to test the ability of compounds to act as free radical scavengers or hydrogen donors. This capability was used to evaluate antioxidant activity. Compounds with radical scavenger capacity are able to reduce DPPH radical using donor hydrogen atom to DPPH free radical based on the type and concentration of sample. Interaction of antioxidant compound with DPPH is based on transfer electron or hydrogen atom to DPPH radical and convert it to 1-1,diphenyl-2-picrylhydrazyl. The result of reduction DPPH radicals causes discoloration from purple color to yellow pale color which indicates the scavenging activity. The decrease of absorbance of DPPH radicals was measured at 515 nm. The result of antioxidant DPPH from *M. oleifera* various extracts is shown at Fig. 1.

The percentage of DPPH inhibition of various extracts; MeOH, ethyl acetate, dichloromethane, and *n*-hexane were 90.59 ± 0.39, 36.19 ± 0.55, 17.67 ± 0.53, 22.45 ± 0.22, respectively, while trolox as a standart had antioxidant activity of 96.61 ± 0.02. The $IC_{50}$ value for *M. oleifera* extracts determined by linear regression. $IC_{50}$ value for the $IC_{50}$ value of methanol extracts was 49.30 µg/mL, dichloromethane extract was 1035.57 µg/mL, ethyl acetate extract was 444.10 µg/mL, *n*-hexane extract was 715.21 µg/mL. The activity of *M. oleifera* methanol extract as shown in Fig. 2. The methanol extract had the highest antioxidant activity among the other leaves extract due to these extract may contain many phenolic compounds that contributed of antioxidant activity. Trolox was used as positive control of antioxidant with $IC_{50}$ 5.89 µg/mL.

### ABTS Radical Scavenging Assay

ABTS is frequently used by the food industry and also agricultural researchers to measure the antioxidant capacities of foods. ABTS assay is used to measure the relative ability of antioxidant to scavenge the ABTS with compared with Trolox standard. The ABTS solution was prepared by reacting a strong oxidizing agent potassium persulfate with ABTS salt. Reduction of blue-green ABTS radical colored solution is measured in long wave 734 nm absorption spectrum. The blue ABTS radical cation was converted back to colorless neutral form during this reaction. Fig. 3 showed the ABTS scavenging activity of *M. oleifera* leaves extracts.

The percentage of ABTS inhibition of various extracts MeOH, ethyl acetate, dichloromethane, and

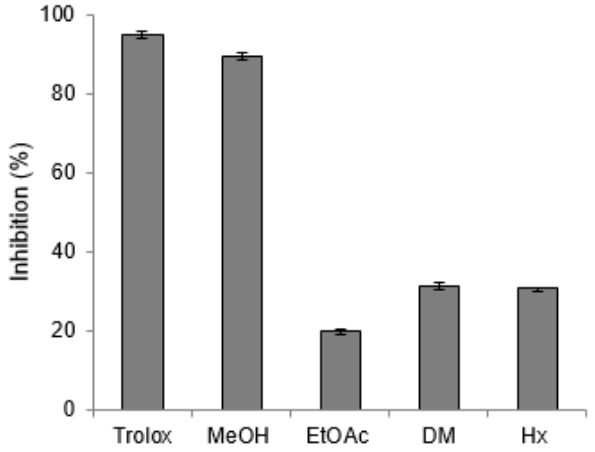


**Fig 3.** ABTS inhibition of *M. oleifera* extracts at a concentration of 99.00 µg/mL; values are mean ± SD of three independent experiments in triplicate at each concentration. MeOH, methanol extract; EtOAc, ethyl acetate extract; DM, dichloromethane extract; Hx, hexane extract

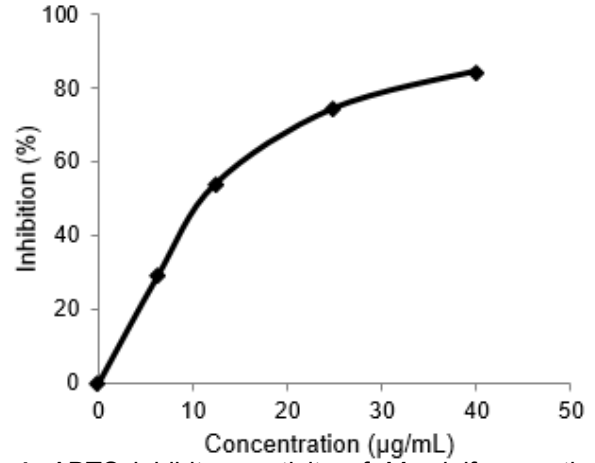


**Fig 4.** ABTS inhibitory activity of *M. oleifera* methanol extract

**Table 1.** Antioxidant activity of *M. oleifera* extracts

| Samples | $IC_{50}$ DPPH (µg/mL) | $IC_{50}$ ABTS (µg/mL) |
|---|---|---|
| Methanol extract | 49.30 | 11.73 |
| Ethyl acetate extract | 444.10 | 241.33 |
| Dichloromethane extract | 1035.57 | 159.06 |
| *n*-hexane extract | 715.21 | 163.79 |
| Trolox | 5.89 | 3.06 |

*n*-hexane were 89.40 ± 0.73, 20.0 ± 40.83, 31.37 ± 0.57, 30.94 ± 1.07, respectively, while trolox as a standard had antioxidant activity of 94.99 ± 0.58. The methanol extract had the highest activity among the other leaves extract.

The $IC_{50}$ value for *M. oleifera* extracts have been determined as shown in Fig. 4, and it gave $IC_{50}$ value of 11.73 µg/mL. Trolox was used as standard antioxidant with $IC_{50}$ 3.06 µg/mL. $IC_{50}$ value for the dichloromethane extract was 159.06 µg/mL, ethyl acetate extract was 241.33 µg/mL, *n*-hexane extract was 163.79 µg/mL (Table 1). The extract can be said has active antioxidant, if $IC_{50}$ value of extract ≤ 100 µg/mL, whereas inactive antioxidant if $IC_{50}$ value of extract ≥ 200 µg/mL [15].

MeOH extract was exhibited fine scavenging abilities against DPPH and ABTS radicals with concentration dependent manner. Thus, the MeOH extract was exhibited a high ABTS radical scavenging efficiency rather than the DPPH radicals, which could be indicated to a different mechanism. In the DPPH assay, the scavenging action may be due to the hydrogen donating ability, whereas scavenging of ABTS radical is due to scavenging of proton radicals induced through donation of electrons [16].

The antioxidant activity of DPPH and ABTS from *M. oleifera* extracts is depends on the solvent used in the extraction. The different compounds can be extracted with different solvent due to different solubility. There is correlation between antioxidant activities with total phenolic compound. MeOH extract should have total phenolic compound higher than the other extracts. The polyphenol compounds such us quercetin and kaempferol should be exist in the leaves [10]. Methanol is the highest polar solvent among the others solvent which can pull out more polyphenol compounds. The previous study reported that methanol extract of *C. spinosa* buds showed result of rich in flavonoids including several quercetin and kaempferol glycosides. They demonstrated to possess strong antioxidant / free radical scavenging effectiveness [17]. Further investigation about toxicity and *in vivo* assay for clinical purposes is needed for clarify the safety of *M. oleifera* methanol extracts.

## CONCLUSION

Antioxidant activies of *M. oleifera* extracts with various solvents (methanol, ethyl acetate, dichloromethane, and *n*-hexane) were determined using DPPH and ABTS methods. MeOH extracts showed the highest antioxidant activity both in DPPH free radical scavenging and ABTS assay *in vitro*. This finding provides scientific evidence for the Indonesian traditional people way, which used *M. oleifera* leaves as one of nutrition food to prevent diseases. This study also indicated that *M. oleifera* leaves can be used as antioxidant source.

**ACKNOWLEDGEMENT**

This work was supported by a grant from research project for international research collaboration and scientific publication 2014, no of contract 07555.13/IT2.7/PN.01.00/2014, Directorate General of Higher Education, Ministry of Education and Culture, Indonesia.

**REFERENCES**

1. Halliwell, B., 2008, *Arch. Biochem. Biophys.*, 476 (2), 107–112.
2. Johnson, I.T., 2007, *Proc. Nutr. Soc.*, 66 (2), 207–215.
3. Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A., and Saija, A., 2005, *Food Chem.*, 89 (4), 549–554.
4. Karadag, A., Ozcelik, B., and Saner, S., 2009, *Food Anal. Methods*, 2, 41–60.
5. Martysiak-Żurowska, D., and Wenta, W., 2012, *Acta Sci. Pol. Technol. Aliment.*, 11 (1), 83–89.
6. Mbikay, M., 2012, *Front. Pharmacol.*, 3, 1–12.
7. Farooq, F., Rai, M., Tiwari, A., Khan, A.A., and Farooq, S., 2012, *J. Med. Plants Res.*, 6 (27), 4368–4374.
8. Arabshahi, D.S., Devi, D.V., and Urooj, A., 2007, *Food Chem.*, 100 (3), 100–1105.
9. Fuglie, L.J., 2001, "The Miracle Tree: Moringa oleifera: Natural Nutrition for the Tropics" in *The Miracle Tree: The Multiple Attributes of Moringa*, CTA Publisher, 172.
10. Charoensin, S., 2014, *J. Med. Plant Res.*, 8 (7), 318–325.
11. Brand-Williams, W., Cuvelier, M. E. and Berset, C., 1995, *LWT Food Sci. Technol.*, 28 (1), 25–30.
12. Dudonńe, S., Vitrac, X., Coutière, P., Woillez, M. and Mérillon, J. M., 2009, *J. Agric. Food Chem.*, 57 (5), 1768–1774.
13. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C., 1999, *Free Radic. Biol. Med.*, 26 (9-10), 1231–1237.
14. Santos, A.F., Argolo, A.C., Paiva, P.M., and Coelho, L.C., 2012, *Phytother. Res.*, 26 (9), 1366–1370.
15. Lisdawati, V., and Kardono, B.S., 2006, *Media Litbang Kesehatan*, 16 (4), 1–7.
16. Chu, Y.H., Chang, C.L., and Hsu, H.F., 2000, *J. Sci. Food Agric.*, 80 (5), 561–566.
17. Bhoyar, M.S., Mishra, G.P., Naik, P.K., and Srivastava, R.B., 2011, *Aust. J. Crop Sci.*, 5 (7), 912–919.