Free radical scavenging activity of methanolic extract of temurui (Murraya koenigii L. Spreng) collected from Langsa, Aceh

Rahayu, S Ningsih, F G Nehru, U Amna and Halimatussakdiah
Chemistry Department, Faculty of Engineering, Universitas Samudra, Langsa, Aceh, Indonesia
E-mail: halimatussakdiah@unsam.ac.id

Abstract. Free Radicals are substances that derive from incompletely oxidized compounds that can damage cells and cause inflammatory diseases, atherosclerosis, cancer, and aging. Plants are known as a source of medicines that are being developed by researchers, such as temurui (Murraya koenigii L. Spreng) which collected from Langsa, Aceh. Temurui or curry leaves, a plant that is usually used as a cooking spice by Aceh people, were extracted using maceration method with n-hexane, ethyl acetate, and methanol, respectively. Methanolic extract of temurui showed very strong DPPH scavenging activity with the IC$_{50}$ value of 77.818 ppm. This activity was contributed by secondary metabolites contained in that plant, such as alkaloids, terpenoids, saponins, flavonoids, and tannins. This plant have great potent to develop as new drugs, especially new antioxidant drugs.

1. Introduction

Temurui (Murraya koenigii L. Spreng) is one of the plants belonging to the family Rutaceae which is known as curry leaves. M. koenigii L. Spreng plants are widespread in Southeast Asia such as Indonesia included in Aceh province [1]. Temurui leaves are rich in protein, fibre, carbohydrates, minerals, nicotinic acid, vitamin C and carotene. Besides, curry leaves are also rich in secondary metabolites such as carbazole alkaloids with diverse chemical compositions and polyphenol compounds which are generally very good as an antioxidant to scavenge free radicals [2].

Free Radicals are substances that derive from incompletely oxidized compounds that have undergone partial burning and that have in their structure oxygen groups capable of initiating at the surface of the cell membranes or even within the cells aggressive oxidation reactions [3] and causes inflammatory diseases, atherosclerosis, cancer, and premature aging. Generally, natural and synthetic antioxidants are types of antioxidants which develop to treat many diseases. However, synthetic antioxidants have side effects that are not good for health [4]. Therefore, a new solution is needed by utilizing natural plants that can reduce free radicals by giving one of their electrons to produce neutral molecules that are not harmful to the human body, such as M. koenigii L. Spreng which is can be developed as antioxidants.

Antioxidants are substances that can release, inhibit, and prevent the oxidation process [5] to neutralize free radicals activities [6]. Antioxidants can improve skin hydration, elasticity and sebum production and improve the physiological abilities of the skin in counteracting skin damage caused by...
free radicals and UV rays [7]. One of the plants that can be used as an antioxidant is curry leaves (*M. koenigii*) or often known as "Temurui" in Aceh region. This plant is known as one of the high antioxidant producing plants (phytoantioxidant) based on previous studies. This plant is commonly found in Aceh Province. The majority of Acehnese use *M. koenigii* L. Spreng as a spice flavoring dish. Traditionally, *M. koenigii* L. Spreng is also used as a treatment for rheumatism, medicine for wounds, dysentery, diarrhea, and snake bites [8]. The chemical contents contained in *M. koenigii* L. Spreng leaves have benefits as bioactive compounds, including antibacterial, antilarvicidal, antimicrobial and antioxidant [9]. Based on the literatures, it is necessary to find out the antioxidant activities of the leaves of *M. koenigii* L. Spreng collected in Langsa, Aceh.

2. Materials and methods

2.1. Plant material

Samples of *M. koenigii* L. Spreng leaves collected in Langsa, Aceh, Indonesia in February 2018.

2.2. Instrument

Rotary evaporator (Buchi R-100) and spectrophotometry Ultraviolet (AE-S60-2UP UV) are used as an instrument in the present study.

2.3. Preparation of extract

The leaves of *M. koenigii* L. Spreng (1 kg) were cut into small pieces and air-dried. The sample was macerated using n-hexane for $3 \times 24$ h, then filtered, and repeated until the filtrate was clear to give n-hexane extract. The extraction process was repeated using ethyl acetate and methanol to yield ethyl acetate and methanol extracts, respectively. In this study, we only reported the methanol extracts.

2.4. DPPH radical scavenging activity assay

The free radical 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was used to determine the free radical scavenging activity of the methanol extract of *M. koenigii* at concentration of 25, 50, and 100 ppm using the spectrophotometric method. As a comparison, the antioxidant activity of vitamin C was tested with concentration of 3, 6, 9, 12 and 15 ppm. Different concentrations of methanol extract and vitamin C (4 mL) were put in the test tube and then 1 mL of a methanolic solution of 0.4 mM DPPH was added. The reaction mixtures were homogenized using a vortex mixer and incubated at 37ºC in the dark for 30 min. The absorbances of test mixtures were read at 517 nm against a blank. Scavenging activity was calculated using the equation (1).

\[
\text{DPPH Scavenging (\%)} = \left(1 - \frac{A_s}{A_o}\right) \times 100
\]  

where, $A_o$ is the absorbance of the blank (DPPH) and $A_s$ is the absorbance of the sample. The inhibitory concentration of the extract that caused 50% inhibition (IC$_{50}$) was calculated by equation $Y = a + bX$ which obtained from the intersection of lines between % inhibition and the concentration [10].

3. Results and discussion

Antioxidant activity was carried out using DPPH method. DPPH is free radical which will react with antioxidant compounds. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) will be changed to 2,2-diphenyl-1-picrylhydrazine (DPPH-H) which is non-radical. An increase in the amount of DPPH-H is indicated by the change in dark purple to pale pink or yellow which is directly proportional to the concentration of extract (qualitative analysis). This enhancement can be observed using a spectrophotometer, and the free radical scavenging activity of sample can be determined (quantitative analysis). The antioxidant activity of methanolic extract of *M. koenigii* L. Spreng leaves with DPPH can be seen in Figure 1. This activity was contributed by secondary metabolites contained in that plant. Phytochemical screening of
M. koenigii leaves has reported previously [11], those are alkaloids, terpenoids, saponins, flavonoids, and tannins. Based on Figure 1, the regression line equation is used to calculate the IC_{50} value of methanol extract of M. koenigii leaves and comparing to the IC_{50} value of vitamin C. The IC_{50} values can be seen in Table 1.

**Table 1.** Antioxidant activity of methanol extract of M. koenigii L. Spreng.

| Sample          | Concentration (ppm) | Absorbance | % Inhibition | IC_{50} (ppm) |
|-----------------|---------------------|------------|--------------|---------------|
| Methanol Extract| 25                  | 0.599      | 33.30        |               |
|                 | 50                  | 0.517      | 42.43        | 77.818        |
|                 | 100                 | 0.391      | 56.46        |               |
| Vitamin C       | 3                   | 0.709      | 24.41        |               |
|                 | 6                   | 0.491      | 47.65        |               |
|                 | 9                   | 0.179      | 80.92        | 6.073         |
|                 | 12                  | 0.065      | 93.07        |               |
|                 | 15                  | 0.055      | 94.14        |               |

**Table 2.** Intensity of antioxidant activities [12].

| Intensity      | IC_{50} (ppm) |
|----------------|---------------|
| Very strong    | < 150         |
| Strong         | 150 - 300     |
| Medium         | 300 - 400     |
| Weak           | 400 - 500     |

Table 1 showed that the methanol extract of M. koenigii L. Spreng leaves obtained the IC_{50} value of 77.818 ppm. Based on Table 2 reported by Mangsaka et al. [12], antioxidant activity of methanol extract of temurui leaves have a very strong free radical scavenging activity, so it can inhibit the occurrence of free radicals. Antioxidant activity is closely related to the content of secondary metabolites, especially phenolic compounds in M. koenigii. Phenolic compounds are able to donate hydrogen atoms to DPPH to form stable compounds (DPPH-H). The higher the phenolic content, the more DPPH radicals react so that the concentration decreases which indicated the higher of antioxidant activity [13]. Previous study [11] has reported that M. koenigii contained secondary metabolites, those are alkaloids, terpenoids, saponin, flavonoids, and tannins. Flavonoids and tannins are the phenolic compounds.
4. Conclusion
The methanol extract of *M. koenigii* leaves has a very strong free radical scavenging activity with an IC$_{50}$ value of 77.818 ppm. The results of this study indicate that the methanol extract of temurui leaves can inhibit the occurrence of free radicals. The antioxidant activity was contributed by secondary metabolites such as alkaloids, terpenoids, saponins, flavonoids, and tannins, especially phenolic compounds such as flavonoids and tannins. Therefore, it can be used as a reference for further research in developing new natural antioxidants.

References

[1] Fachraniah, Kurniasih E and Novilasi D T 2012 *Jurnal Reaksi* (J. Sci. Tech.) 10 (21)
[2] Kamat N, Pearline D and Thiagarajan P 2015 *J. Pharm. Biol. Chem. Sci.* 6 (5)
[3] Butnariu M and Samfira I 2012 *J. Bioequiv. Availab.* 4 iv-vi
[4] Gazali M, Nufus H, Nurjannah and Zuriat 2017 *JPHPI* 22 (1) 155-163
[5] Rumengan A P and Mantiri D A 2015 *Jurnal LPPM Bidang Sains dan Teknologi* 2 (2) 71-77
[6] Istiningrum R, Amin M and Lestari U 2016 *Prediksi kandidat protein target senyawa alami anti-aging scopoletin dari Morinda citrifolia secara in silico, Seminar Nasional Pendidikan dan Saintek* 193-196
[7] Sayuti N A 2017 *Jurnal Kebidanan dan Kesehatan Tradisional* 2 (1) 1-5
[8] Utami Y, Pupitasari E and Pangaribowo D A 2015 *Artikel Ilmiah Hasil Penelitian Mahasiswa* 1-5
[9] Mustanir, Al-Qarana T R, Gusvianna H and Saidi N 2019 Analisa potensi ekstrak daun karis (Murraya koenigii L. Spreng) *TM Conference Series* 02 1-8
[10] Ginting B, Mustanir, Helwati H, Desiyania L S, Eralisa and Mujahid R 2017 *Jurnal Natural* 17 (1) 39-44
[11] Sukma F F, Sahara D, Ilhsan F N, Halimatussakdiah, Wahyuningsih P and Amna U 2018 *Jurnal Jeumpa* 5 (1) 34-39
[12] Mangkasa M Y, Rorong J A and Wuntu A D 2018 *Jurnal Ilmiah Farmasi –UNSRAT* 7 (4)12-22
[13] Adawiah, Sukandar, D dan Muawanah A 2015 *Jurnal Kimia VALENSI: Jurnal Penelitian dan Pengembangan Ilmu Kimia* 1 (2) 130-136

Acknowledgments
We would like to thank Belmawa (Kemenristekdikti) for the Grant of PKM-P and Universitas Samudra for the technical supports.