The experiment of activity and stability of antioxidant extracted from Senduduk (Melastoma malabathricum L) leaves at various conditions of concentration, pH values, and temperatures

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Abstract. Melastoma Malabathricum L. (Senduduk) is a type of weed that serves as an antibacterial, antioxidant, anti-inflammatory, anti-cancer, anti-hepatoxic, anti-diabetic and antiseptic to kill or prevent the growth of microorganisms. The purpose of this study is to obtain antioxidant extracts from Senduduk leaves and to test its antioxidants stability in various temperatures and pH. Senduduk leaves are extracted by macerating them with 95% ethanol solvent for 72 hours, and then the solvent is evaporated using a vacuum evaporator at 40 °C. In this experiment, DPPH (1,1-diphenyl-2-pikrihidazil 1,1-diphenyl-2-pikrihidazil) method is used to test the antioxidant activity. Based on this experiment, the extract yield produced from Senduduk leaves was 10.40% (w/w). The results show that the extracts experience an ability to inhibit DPPH free radical formation by 91.734% at 100 ppm concentration. It is known that the antioxidant from the extract of Senduduk leaves is stable at pH 3-5 and at temperature of 70 °C. The IC50 (Inhibition Concentration) value is 55.432 ppm, which proves that the antioxidant properties of the leaves extracts can be classified as strong antioxidant.

Keywords: Antioxidant activity, stability, DPPH, Melastoma malabathricum L

1. Introduction
Natural antioxidants can be derived from endogenous Indonesia plant. Thousand kinds of leaves, stems, seeds, fruits, roots, flowers and so on are already identified as antioxidants sources. The use of these natural antioxidants is also applied commercially in form of one or more compounds for food processing and food additives [1]. A research done by [2] has extracted antioxidant compound from rosella flower and resulted on antioxidant activity as much as 293.09 µL/mL for IC50. Antioxidant components that exist in nature have a different chemical structure. In general, these compounds are amino acids, carotene, sinarmat acids, flavonoids, melaniodin, certain organic acids, reducing agents, peptides, phosphatides, polyphenols, tannins and tocopherols [3]. Vitamin C and anthocyanin [2] . Most of the natural antioxidants are derived from plants that are isolated to obtain flavonoids and other phenolic compounds [4]. Flavonoids are phenolic compounds obtained from the synthesis plant that are present in all parts of the plant such as fruit, leaves, wood and bark.
Senduduk is a plant that grows wild in places that receive enough sunlight, such as shrubs or on the slopes and can grow in area 1,650 m above sea level [5]. Senduduk plants are widespread in several islands in Indonesia, in Sumatra, Java, Irian Jaya and Kalimantan [6]. These herbs are efficacious for treating diarrhea, vaginal discharge, burns, ulcers, uterine bleeding, ulcers and bleeding wounds [7]. Phytochemical screening results show that the leaf Senduduk contains some secondary metabolites such as flavonoids and phenolic compounds that can be used for treatment as anticancer and antioxidant [8]. Senduduk leaf also contains tannin, steroid, saponins, and glycosides which serves to kill or inhibit the growth of microorganisms [9]. Previous phytochemical studies also revealed that the plant Senduduk contains b-sitosterol-3-OBD-glucopyranoside, quercetin, and rutin quercitrin. Therefore, the active compounds or secondary metabolites owned by the Senduduk leaves are potential as antioxidants

Antioxidants are descriptors of compounds that have antioxidant activity. The compound can prevent a variety of diseases caused by free radicals or reactive oxygen species (ROS) [10]. Free radicals are atoms or molecules with an unpaired electron in their outer orbitals and reactive [11]. The antioxidant compounds can donate electrons to the free radicals that free radical molecules become reactive and unstable [12]. Natural antioxidant is applied as food additives, bioactive nutraceuticals and biopharmaceuticals on daily activities. Related to the extraction, characterization and utilization of natural antioxidant are intensively performed to find potential candidates against the aging process [13].

The potential possessed by Senduduk provides opportunities for further testing to be developed in the world of food packaging in preventing the free radicals that can damage the food. One study that has tested the antioxidant activity of the extracts of leaves Senduduk is the research conducted by [14]. The study proves that Senduduk leaf extract has potent antioxidant activity measured by DPPH and correlate to the levels of total phenolic and flavonoid. Testing antioxidant activity using DPPH method has the advantages of which are stable, do not form dimers due to delocalization of the free electrons in the molecule, and does not require the entire substrate so it is much simpler with a faster analysis time. This study is therefore undertaken to evaluate the effects of an ethanol extract of the leaves of Melastoma Malabathricum L as a source of natural antioxidants in antioxidant making films to maintain color in fresh meat.

2. Materials and methods
The materials used in this research ware Melastoma Malabathricum L leaves, water, aquades, DPPH (1,1-diphenyl-2-pikrihidazil 1,1-diphenyl-2-pikrihidazil), ethanol. The tools used were measuring cups, cup glasses, pH meters, and spectrophotometers. This study includes extraction of M. Malabathricum leaves and temperature stability test and pH M. Malabathricum of extract.

2.1. Materials
Senduduk (M. Malabathricum) leaves were collected in February 2019 from the health forest in IPB University, Bogor, Indonesia. The samples were washed with cleaned water to remove the extemporaneous matter and dried at 40 °C for 72 hours. The samples were ground into powder form (40 mesh) by miller.

2.2. Preparation of extracts
Approximately 50 gr of dried powder M. Malabathricum leaves were weighed and placed into a round bottom beaker glass with 500 ml (1 : 10 w/v) of the extracting solvent. The sample was extracted using a maceration extraction method with ethanol (EthOH) 95% as a solvent for 72 hours. Extracts were decanted and filtered through Whatman filter paper no. 1. The filtrate was then concentrated in a rotary evaporator at 40 °C with reduced pressure. The extracts were dried at 40 °C and kept in a freezer at 4 °C for further use.
2.3. Determination of percentage yield (%)
The percentage yield of the extract was determined gravimetrically using the dry weight of extract (x) and soaked samples material (y) using this formula:

\[
\text{Percentage yield} = \frac{x}{y} \times 100
\]  
(1)

The extraction yield was calculated for each extract and results were presented as a percentage yield (%).

2.4. Determination of DPPH radical scavenging activity (antioxidant)
DPPH radical scavenging activity of the *M. Malabathricum* leaves was estimated by adopting the method [15] with minor modification. A 2 mL of extract at different concentrations (100, 50, 25, 12.5, 6.25, and 0 mg/mL), was added in 18 well micro plate and 3 mL of 0.4 mM solution of DPPH was added to the mixture in wells. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm using microplate reader. Each sample was assayed in triplicate and then mean values and SD were calculated. The percentage of inhibition of free radicals was calculated. The IC50 values were calculated as the concentration of a test sample required to give 50% radical scavenging activity (DPPH).

\[
\text{Percentage inhibition (\%)} = \left(\frac{A\text{ control} - A\text{ sample}}{A\text{ control}}\right) \times 100
\]  
(2)

2.5. Antioxidant stability at temperature and pH
The antioxidant stability testing of pH treatment was carried out by taking extracts of 10 mL each then adding phosphate-citrate buffer solution according to the desired pH namely (3, 4, 5, 6, 7, and 8) then stirred ± 5 minutes. At the temperature treatment, extracts were taken as much as 30 mL each, then put into a test tube and then heated at a temperature of 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C for ± 15 minutes. Then the antioxidant activity was measured with the DPPH method. The measurement data obtained are then presented in graphical form.

3. Result
3.1. Determination of percentage yield (%)
The extractable matter of ethanol extract of each sample is presented in table 1; the amount of extract from the crude powdered drug is determined and presented. The percentage yield is calculated.

| Sample       | Percentage yield (\%)(w/w) |
|--------------|----------------------------|
| Leaf         | 10.40                      |

Table 1. Percentage yield of ethanol extract of *M. Malabathricum* leaves.

The yield of extract is calculated based on the final weight ratio (dry weight of extract) with initial weight (soaked samples of material) multiplied by 100%. The above table shows that the percentage yield of *M. Malabathricum* leaf extract is 95% with ethanol solvent for 72 hours and is equal to 10.4% on the basis of dry sample weight. The results are higher than the results of research conducted [16] which obtained yield of 6.82%, by using a soaking time of 48 hours. The longer extraction time causes the warming effect to be longer and the better the chance of solvent to have contact with the greater material so that it will grow to the point of saturation [17].
3.2. Determination of DPPH radical scavenging activity (antioxidant)

DPPH radical has been used to determine free radical scavenging antioxidant activities of pure compounds such as fruit and plant extracts and also food materials [18]. Radical scavenging activity is expressed as percentage according to the following formula:

\[
\% \text{ DPPH radical scavenging activity} = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100
\]  

(3)

The ability of ethanol extracts of leaves of *M. Malabathricum* to donate hydrogen and act as an antioxidant was studied using the DPPH radical scavenging method. Data processing techniques were performed by comparing the concentration with the value % antioxidant activity of each sample in a regression graph. The test results using DPPH antioxidant can be seen in table 2.

| Concentration (ppm) | Absorbance Control | Absorbance Sample | Antioxidant Activity (%) |
|---------------------|--------------------|-------------------|--------------------------|
| 100                 | 0.738              | 0.061             | 91.734                   |
| 50                  | 0.738              | 0.454             | 38.482                   |
| 25                  | 0.738              | 0.523             | 29.132                   |
| 12.5                | 0.738              | 0.649             | 12.059                   |
| 6.25                | 0.738              | 0.693             | 6.0975                   |

The data in table 2 show various concentrations as the value of (X) and the % of antioxidants as the value of (Y). From figure 1 which has been plotted, obtained equation as shown in the figure was \( Y = 0.902X \). The equation was used to find an effective concentration of the extract to reduce free radicals DPPH or IC50.

![Figure 1. Antioxidant activity at various concentration.](image)

IC50 is an effective concentration of extract needed to drown out 50% of the total DPPH, so that the value 50 is substituted for the value of Y. After substituting the value 50 to the value of Y, you get the value of X as the IC50 value. Based on these data, the IC50 value of all samples tested on dilution variation indicate that IC50 values obtained amounted to 55.432 ppm. IC50 values are in accordance with the parameters in table 3, showing that the leaf extract of *M. Malabathricum* is a strong antioxidant (IC50 values ranging between 50-100 ppm).
Table 3. Antioxidant properties based on IC50 value [14].

| IC50     | Antioxidant Properties |
|----------|------------------------|
| 50 ppm   | Very Strong            |
| 50-100 ppm| Strong                 |
| 100-150 ppm| Moderate              |
| 150-200 ppm| Weak                  |

3.3. Antioxidant stability at pH and temperature

Leaf extract of *M. Malabathricum* has been dissolved in distilled water with a concentration of 100 ppm and then was treated with various pH by adding a solution of phosphate buffer citrate according to the desired pH. There were (2, 3, 4, 5, 6, 7, and 8) then stirred for ± 5 minutes and allowed to stand for 30 minutes. The mixture was then measured for the antioxidant activity by DPPH method. The experimental results are shown in table 4.

Table 4. Antioxidant stability of *M. Malabathricum* leaves extracts against pH.

| pH | Absorbance Control | Absorbance Sample | Antioxidant Activity (%) |
|----|--------------------|-------------------|--------------------------|
| 3  | 0.68               | 0.31425           | 53.786                   |
| 4  | 0.68               | 0.34100           | 49.852                   |
| 5  | 0.68               | 0.36525           | 46.286                   |
| 6  | 0.68               | 0.40925           | 39.816                   |
| 7  | 0.68               | 0.49625           | 27.022                   |
| 8  | 0.68               | 0.60275           | 11.360                   |

According to the table above the curve, it is known that the larger (alkaline) pH values tested, the more antioxidant activity decreases. This may be due to the fact that buffer solution made of citric acid is pretty good because of high water solubility. In industry, citric acid is not only used as a pH regulator, but it is also used as flavor enhancers, preservatives, preventing damage to the color and aroma, keeping the carbonation, keeping turbidity, as antioxidants, and flavoring cold [20]. The curve above also shows that the antioxidant activity of leaf extract of Senduduk is more stable at acidic pH. Other studies also conducted by [21] that rosella extract at pH 2-4 has an antioxidant activity of around 70%. The higher the pH, the greater the decrease in antioxidant activity to 30% at pH 8. This is because according to [22], as pH increases, the concentration of hydrogen ions in the medium decreases so that the release of hydrogen ions begins by phenolic compounds (antioxidants). This results in higher pH, and antioxidant protection by phenolic compounds decreases.
In the temperature treatment, each extract of 30 mL was heated for 15 minutes at various temperatures. The percentage of Senduduk leaf extract antioxidant activity was measured with DPPH. Data on the stability of the antioxidant activity at various temperatures are shown in table 5.

Table 5. Antioxidant stability of *M. Malabathricum* leaves extracts at various temperatures.

| Temperature (°C) | Absorbance Control | Absorbance Sample | Antioxidant Activity (%) |
|------------------|--------------------|-------------------|--------------------------|
| 40               | 0.8                | 0.095             | 88.125                   |
| 50               | 0.8                | 0.087             | 89.125                   |
| 60               | 0.8                | 0.084             | 89.500                   |
| 70               | 0.8                | 0.077             | 90.375                   |
| 80               | 0.8                | 0.081             | 89.875                   |

Based on the curve, it is known that there is an increase in antioxidant activity at a temperature to 70 °C that is equal to 90.375% and then decreased antioxidant activity at a temperature of 80 °C to be 89.875%. Antioxidant extract from the leaves Senduduk is more stable at pH 3-5 and at a temperature of 70 °C. Previous research has also been conducted [23] which states that the crude extract of onion leaves contains phenolic and flavonoid components that can affect the activity of antioxidants. It is known that the antioxidant activity of the extract is not stable to heat and pH. The crude extract of onion is in more stable conditions of 70 °C temperature and pH 4-5.

4. Conclusion
The present study has proved that the extracts of leaves of *M. Malabathricum* plants possess antioxidant properties. The results show that the ethanol extracts of Senduduk leaves display an ability to inhibit DPPH free radical formation by 91.734% at 100 ppm concentration. The antioxidant extracted from the leaves of Senduduk is stable at pH 3-5 and at temperature of 70 °C. The IC50 value obtained is 55.432 ppm, showing that the antioxidant properties of the leaf extracts can be classified as strong antioxidant.

5. References
[1] Pratt DE And BJF Hudson 1990 *Natural Antioxidant not Exploited commercially* In: Hudson BJF (Ed.) Food Antioxidant (London: Elsier Applied Science)
[2] Purbowati IS, Syamsu K, Warsiki E, Sri HR 2016 Optimization of phenols extraction from roselle (*hibiscus sabdariffa*) by microwave assisted extraction as antibacterial and antioxidant agents. *Journal of Agroindustrial Technology* 26 23-30
[3] Dugan LR 1985 *Natural Antioxidant In: Simic MG and Karel* (Eds.) Autoxidation In Food and Biological System (New York and London: Plennum Press)
[4] Triantaphyllou K, Blekas G and Boskou, D 2001 *Int. J. Food Sci. Nutr.* 52 313-7
Advancements in the extraction and evaluation of biological activities of medicinal plants from Indonesia

[5] Dalimartha S 2000 *Atlas Tumbuhan Obat Indonesia* Jilid I (Jakarta: Trubus Agriwidya)
[6] Ghobhi D 2009 Inhibition potential of *Melastoma malabathricum* L. leaves against *Trichophyton mentagrophytes* and *Candida albicans* Berita Biologi 9 523-7
[7] Djauhariya E and Hernani 2004 *Gulma Berkhasiat Obat* (Jakarta: Seri Agrisehat) p 74-75.
[8] Zakaria ZA, Roffe MS, Mohamed AM, Tch LK and Salleh MZ 2011 *J. Acupunct. Meridian Stud.* 4 248-56
[9] Robinson T 1995 *Kandungan Organik Tumbuhan Tinggi* (Terjemahan K. Padmawinata) (Bandung: Penerbit ITB Bandung)
[10] Hostettmann K 2014 *Handbook of Chemical and Biological Analytical Plant Methods* (Manchester: Wiley)
[11] Jethinalalkhosh J P, Antony A, Pravena P and Doss V A 2016 *Asian J. Pharm. Clin. Res.* 9 236-9
[12] Mokoginta E P, Runtuwe M R J and Wehantou F 2013 Pengaruh metode ekstraksi terhadap aktivitas penangkal radikal bebas ekstrak metanol kulit biji pinang yaki (*Areca vestiaria Giseke*) *Pharmacon* 2 109-13
[13] Irawan W K, Murdiyanto, Enos T A, Syafizal and Yong-un K 2014 Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia *FSHW* 3 191-6
[14] Sharma K H and Atul K 2011 *J. Chemistry* 23 434-438
[15] Mis-Salihoglu E, Akaydin G, Caliskan-can E, Yardim-akaydin S. Evaluation of antioxidant activity of various herbal folk medicines. *J. Nutr. Food Sci.* 3 222
[16] Sari N M, H Kuspradini, R Amirta and I W Kusuma 2018 *IOP Conf Series: Earth and Environmental Science* 144(2018) 012029
[17] Diantika F, Sutan S M and Yulianingsih R 2014 *J. Teknologi Pertanian* 3 159-64
[18] Vikas Kumar, Danish Ahmed, Pushpraj S Gupta, Firoz Anwar and Mohd Mujeeb 2013 *BMC Complementary and Alternative Medicine* 13 222
[19] Molyneux P 2004 *J. Science Technology* 26 211-219.
[20] Rufaida R 2008 Proc Seminar PATPI Palembang 14-16 Oktober 2008
[21] Purbowati IS, Syamsu K, Warsiki E, Sri HR 2016 *Stability of phenolic compounds in rosella flowers extract and nanocapsul flowers in various ph, temperature and time variations* 10(1):36
[22] Tensiska, Wijaya CH, Andarwulan N 2003 Antioxidant activity of andaliman fruit extract (*Zanthoxylum acanthopodium* DC) in several food systems and the stability of its activity against temperature and pH conditions *J. Teknol. dan Ind. Pert.* 14 29-39
[23] Siregar T M, Eveline, Felita A J 2015 Proc SNST ke-6 Tahun 2015 ISBN 978-602-99334-4-4

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