Introduction

Free radicals can cause oxidative stress and damage to our cells or tissues [1, 2] causing many diseases including type 2 diabetes mellitus, hypercholesterolemia, neurodegenerative diseases, and cancer [3, 4]. Free radicals are unstable due to their unpaired electrons. Free radicals or oxidants bind to other electrons by forming a new free radical in the oxidation reaction process [1]. Unpaired electrons react with other substances such as proteins, fats, and DNA in the human body [2]. Antioxidants can inhibit oxidation reactions with chemical structures that have a hydroxyl group on the flavone ring. Antioxidant compounds are essential for our body to neutralize and prevent the effects of free radical compounds [3, 4].

Flavonoid compounds are antioxidants [3]. Recent research states that flavonoids can improve the immune system in preventing degenerative diseases and infectious diseases by viruses, such as COVID-19 [5]. In addition to antioxidant activity, flavonoid compounds also have antiviral activity that can potentially prevent severe illnesses from viral diseases like COVID-19 [6].

The COVID-19 virus directly affects the immune system which can trigger various diseases, inflammatory, and infectious complications of the virus [7]. Inflammation occurs characterized by white blood cells that will respond to the production of cytokines. Cytokines will bind to cell receptors so that they can trigger inflammation [8]. According to recent research by Mrityunjaya et al. (2020), quercetin compounds have the potential to be protective against SARS-CoV-2 which may prevent severe illnesses from COVID-19 by reducing inflammation [9]. Furthermore, studies have suggested that quercetin compounds may be able to reduce inflammation caused by COVID-19 and help prevent hospitalization due to COVID-19.

Moringa (Moringa oleifera L.) contains compounds that act as antioxidants. The previous...
research stated that phytochemical screening in Moringa leaf extract contains flavonoid compounds, tannins, terpenoids, alkaloids, and saponins [11]. Moringa leaves contain flavonoid compounds with marker compounds quercetin [12]. This compound can act as an antioxidant because it has a hydroxyl group with the working mechanism of donating a hydrogen atom to a hydroxyl group that can react with free radicals [13]. Moringa leaf ethanol extract has a stronger antioxidant power than n-hexane and ethyl acetate extracts because ethanol is the most effective solvent to attract flavonoid compounds [14]. The World Health Organization estimates that about 80% of the population in the developing countries use herbal plants as traditional medicine for basic needs for the treatment of diseases. The use of herbs as medicines is associated with their ability as antioxidants to be made into pharmaceutical preparations [15]. Dosage forms that can be considered for the formulation of herbal medicines from Moringa leaves are capsules. Capsules have an attractive shape, can mask the smell and even taste of the active substance or added ingredients, are easy to swallow, and disintegrate quickly in the stomach [16]. Our study aims to formulate Moringa leaf extract into capsules for the evaluation of physical properties, determination of total flavonoid content, and test of antioxidant activity using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method.

**Methods**

**Materials**

The ingredients used were Moringa leaf powder obtained from Beringharjo Market, Yogyakarta, 96% ethanol (Bratachem), capsule shell no.0, DPPH (Merck), quercetin (Merck), aerosil, polyvinylpyrrolidone (PVP-K30), avicel, lactose, formic acid, methanol pro analysis, toluene, ethyl acetate, and silica gel GF-254 (Merck). The equipment used is Pyrex glass, Oven (Memmert UN 55), Spectrophotometry (Thermo Scientific 201 UV-Vis), and Camag Thin-Layer Chromatography (TLC) Scanner.

**Collection and extraction procedure**

Moringa leaf powder (M. oleifera L.) was obtained from Beringharjo Market Yogyakarta in September 2020. Moringa leaf powder weighed 500 g dissolved using 96% ethanol 2.5 L (1: 5), maceration for 3 × 24 h, and remuneration 1 time. The filtrate was heated over a water bath or concentrated using a vacuum rotary evaporator 60°C and the thick extract formed will be calculated by calculating the percentage yield [12].

**Formulation**

Moringa leaf extract capsules (MLECs) were made by mixing Moringa leaf ethanol extract with powdered excipients into a mortar and pestle until homogeneous. Then dissolve the polyvinylpyrrolidone with 96% ethanol until it dissolves evenly and mix it into the powder containing the ethanol extract of Moringa leaves in a mortar. The wet granules were then sieved using a number 10 mesh sieve and dried at 40–60°C for 1 h in the oven. The granules were then sieved using a number 12 mesh sieve and then put into capsule shell number 0 [16]. The MLEC formulation is listed in Table 1. Furthermore, evaluation of physical properties includes moisture content (MC) (%), angle of rest (degrees), flow rate (g/s), weight uniformity (g), and breakdown time test (minutes) [17].

**Determination of total flavonoid levels in Moringa leaf capsules by TLC densitometry**

The capsule sample of the best formula from the physical properties test weighed 50.0 mg and then dissolved in pro-analytical methanol (pa) in a 10 mL volumetric flask. The quercetin standard 25.0 mg of was dissolved in 25 mL of pro-analytical methanol solvent. Prepare a series of standard solutions of 20–100 ppm quercetin. The stationary phase using a 10 × 10 cm Silica Gel GF254 plate was heated in an oven at 100°C for 10 min. Samples and standards were spotted (0.5 ml) on the plate, at a distance of 10 mm. The elution distance used was 80 mm carried out at room temperature (28 ± 2°C), with toluene: ethyl acetate: formic acid, 5:4:0.2 (v/v/v) as mobile phase, in a glass Camag chamber previously was saturated with the mobile phase for 20 min. The stationary phase (plate) of TLC which has been dried in a fume hood is then inserted into the Densitometer (Camag TLC Scanner). This research was conducted at the Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta [18].

**Test of antioxidant activity of MLEC with DPPH method**

Preparation of 100 ppm DPPH solution was carried out by weighing 10 mg of DPPH powder then dissolved in 96% ethanol to the mark using a 100 mL volumetric flask and as a mother liquor placed in a dark glass bottle. The blank solution was made using 1.0 mL of 100 ppm DPPH solution then dissolved using 96%...
ethanol in a 5.0 mL volumetric flask, adding solvent to the limit mark. Let stand for 30 min and measure the absorbance at a wavelength of 517 nm. The 100 ppm quercetin mother liquor was prepared by weighing 10 mg of quercetin powder then dissolved in 96% ethanol while stirring and homogenized in a 100 mL volumetric flask to make 100 ppm quercetin solution. Furthermore, variations in concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were made. Solutions of various concentrations as much as 1 ml, each added 1.0 mL of 100 ppm DPPH solution, vortexed, and incubated for 30 min in a dark room [19].

\[
\text{% Reduction} = \left( \frac{\text{Abs control} - \text{Abs assay}}{\text{Abs Control}} \right) \times 100\%
\]

Abs: Absorbance

Equation 1. The equation of DPPH reduction percentage.

The sample solution was made with a concentration of 1000 ppm by weighing the capsule powder according to the calculation of sample preparation and dissolved using 96% ethanol as solvent. Add 96% ethanol to the mark in a 25 mL volumetric flask, shake until homogeneous. Then make a series of sample solution concentrations, namely, 100; 200; 300; 400; and 500 ppm of the mother liquor sample of 1000 ppm. Solutions of various concentrations as much as 1 ml, each added 1.0 mL of 100 ppm DPPH solution, vortexed, and incubated for 30 min in the flask covered.

The DPPH reduction percentage of MLEC (Moringa Leaf Ethanolic Capsule), which was calculated by the Equation 1, that would be followed by the regression line. The IC50 value is calculated by entering the number 50 as Y in the linear regression equation (Y = bx + a) [19].

Results

Evaluation of physical properties

The thick extract formed was calculated by calculating the percentage yield as 23.8%. Table 2 shows the results of the evaluation of Moringa leaf herbal extract capsules by several tests such as the calculation of % MC, granule angle of repose, granule flow rate, capsule weight uniformity, and capsule disintegration time test with several formulas using different amounts of PVP [17].

<table>
<thead>
<tr>
<th>Table 2: Results of evaluation of Moringa leaf herbal extract capsules</th>
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<td>Formula</td>
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<td>I</td>
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Total flavonoid levels in Moringa leaf capsules by TLC densitometry

Spectro-densitometric scanning quercetin was performed in fluorescence 389.0 nm (Figure 1), while at the theory mode at 380 nm [18]. Table 3 shows that MLECs contain a high flavonoid 8.97% (w/w), compared to a thick Moringa leaf extract containing a total flavonoid not <6.30% (w/w) calculated as quercetin. The results of linear regression calculations between the grade series and the area (Figure 2) are obtained with linear regression equations Y = 101.21x – 508.19 with $R^2$ = 0.9849. The linear regression equation can be used to calculate the total flavonoid content as quercetin.

Antioxidant activity of capsule

The standard DPPH experimental wavelength was 517.16 nm. The results of the antioxidant capsules of Moringa leaf extract compared with quercetin. Table 4 shows the antioxidant results in all MLEC formulas and quercetin standards including the category of very strong antioxidants (< 50 ppm) [13].

Discussion

Moringa leaf ethanol extract was obtained using the maceration or filtration method without heating, this method prevents decomposition of the compound due to heating. Immersion using ethanol solvent has better antioxidant activity than n-hexane and ethyl acetate solvent [14]. Maceration with ethanol is a suitable extraction method and solvent for extracting high-quality antioxidant raw materials from Moringa leaves for the development of pharmaceutical and nutraceutical products [20], [21]. The TLC densitometry method in the analysis of the total flavonoid content of Moringa leaf capsules was chosen because it is selective and sensitive when compared to the UV–visible spectrophotometric method. The high-performance liquid chromatography (HPLC) method is also an option in the analysis of flavonoid content, but this method has limited applications, such as extensive sample cleaning and the need for expensive solvents and a longer column stabilization period. High-performance TLC remains the choice of flavonoid content analysis because it is a versatile analytical technique that requires inexpensive instrumentation and expertise [18].

Moringa contains vitamins, minerals, proteins, essential phytochemicals, amino acids, carotenoids, and flavonoids that can be antioxidants [21]. Studies have examined the use of Moringa as a supplement in preventing infection with pathogens including SARS-CoV [22]. The immune modulator and immune stimulator activity of Moringa leaves makes it a suitable natural nutritional supplement and immune enhancer.
against SARS-CoV-2 [23]. A study using an in silico-based approach showed the possibility of identifying a strong inhibitor of SARS-CoV-2 Mpro from plant sources, such as Moringa. The antioxidant properties of the Moringa extract may be able to enhance the immune system of secondary infections after COVID-19. Other components in Moringa leaves that may have beneficial impact on the immune system play a role include pterygospermin and apigenin [24].

Table 3: Total flavonoid capsule formula II

<table>
<thead>
<tr>
<th>Sample replication</th>
<th>AUC</th>
<th>Quercetin levels % (w/w)</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1618.30</td>
<td>8.49</td>
<td>6.97 ± 0.44</td>
</tr>
<tr>
<td>2</td>
<td>1835.77</td>
<td>9.35</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1781.90</td>
<td>9.08</td>
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Das et al. (2020) showed that the total flavonoid content in M. oleifera leaves extract before synthesis was 23.0 ± 0.3% and in M. oleifera leaves extract after synthesis 17.0 ± 0.4%. While in this study, we made the MLEC formula, which had the flavonoid content of 8.97 ± 0.44%. This may be because this study used capsules as a sample so that the flavonoid content is reduced and further studies need to be carried out regarding the optimization of product manufacture on the total flavonoid content [25]. Studies have suggested that the content of flavonoids in Moringa leaves apart from being an antioxidant, may also be used as an antidiabetic, antibacterial, and dietary supplement [26], [27]. Nutraceutical products or supplements containing Moringa leaves may be able to contribute to prevention obesity and type 2 diabetes in Indonesian children and adolescents aged 10–18 years, many of whom lead unhealthy lifestyles with poor dietary choices [28], [29].

In vitro, quercetin when reacted with DPPH, will be able to stabilize the unpaired electrons in DPPH. Chemical studies on quercetin compounds mainly focus on the antioxidant activity of metal ion complexes and their complex ions [30]. Pharmacologically, the antioxidant activity of quercetin compounds mainly its effects on enzymatic activity, glutathione, reactive oxygen species, and signal transduction pathways, which are caused by environmental exposure and toxicological conditions [29], [30]. The compound quercetin exhibits strong antioxidant activity by maintaining oxidative balance [30]. As an antioxidant, quercetin controls the effector mechanism of ROS production, with positive and negative effects of this antioxidant agent under conditions of oxidative stress [30]. Given the high concentration of quercetin in Moringa, the use of its supplements can inhibit the entry of the virus and have immunomodulatory properties. Oral supplementation doses with quercetin up to 1 g/day for 3 months did not produce significant side effects, so the consumption of Moringa leaf supplements was in accordance with this dose [6]. As an antioxidant, quercetin controls the effector mechanism of ROS production, with positive and negative effects of this antioxidant agent under conditions of oxidative stress [31], [32].
Medicinal plants since hundreds or even thousands of years ago have an important role in the treatment of various diseases by humans. The development of drugs from natural ingredients, especially medicinal plants, continues to be researched to provide alternative therapies and disease prevention efforts. Moringa capsules from M. oleifera leaf extract have the potential to be a supplement to maintain health with their flavonoid content. This is also known by the results of the antioxidant activity of Moringa leaf capsules in vitro (DPPH) which showed strong antioxidant activity. It is necessary to test the antioxidant activity of Moringa leaf capsule supplements in humans to determine its pharmacological effectiveness clinically.

Conclusion

All MLEC formulas met the evaluation parameters of physical properties and had very strong antioxidant activity category. Evaluation of the MLEC formula II is the best capsule evaluation parameters and had a very strong antioxidant activity IC50 of 40.2 ppm, and the total flavonoid content was 8.97 ± 0.44% (w/w). The antioxidant properties of MLEC may be useful for the prevention of obesity and type 2 diabetes as well as for improving the immune system. We suggest future studies to examine the effectiveness of Moringa supplements in preventing severe illnesses from viral diseases like COVID-19.

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References


