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Antioxidant Activity from Combination of *Centella asiatica* Herb Extract and *Moringa oleifera* Leaves Extract

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ABSTRACT

Antioxidants have an important role in inhibiting free radicals, where free radicals can cause a hereditary disease. Natural medicines that are efficacious as an antioxidant are Gotu kola and kelor leaves. This study aimed to obtain the potential antioxidant compounds in Gotu kola herb extract, kelor leaves extract and the combination using the DPPH method. This research was started by extracting simplicia with 96% ethanol, followed by preparing combination of Gotu kola extract and moringa extract ratio (1:1), (1:2), (1:3), (2:1), (3:1) which was carried out at a concentration of 20, 40, 60, 80, and 100 µl/mL. Furthermore, the antioxidant activity was tested by UV Vis spectrophotometry. Results showed that the IC_{50} value for the Gotu kola herb extract was 76.66 ± 0.56. Kelor leaves extract 82.66 ± 2.32, a combination of Gotu kola: Moringa (1:1) 74.07 ± 1.16, a combination of Gotu kola: Moringa (1:2) 86.56 ± 1.11, a combination of Gotu kola: Moringa (1:3) 65.09 ± 3.03, a combination of Gotu kola: Moringa (2:1) 56.65 ± 1.61, a combination of Gotu kola: Moringa (3:1) 67.58 ± 2.77. The best potential antioxidant activity was showed in the combination of Gotu kola: Moringa (2:1).

Keywords: Antioxidant; Centella asiatica; DPPH; Moringa oleifera.

INTRODUCTION

Free radicals are a term that has long been identified each inside the global of medicine and health, that is because of the unpaired electrons in the outer orbit become reactive free radicals, triggering premature aging, carcinoma, artheosceloris and diabetes (Khaira, 2010). Prolonged exposure to free radicals is harmful to health. Intake of antioxidants can help eliminate free radicals to maintain a healthy body (Yuliani, 2015). Free radicals can damage cells and trigger various types of diseases and antioxidants have the potential to prevent free radicals in the body, which are feared to damage cells and cause various diseases (Werdhasari, 2014; Meliana, 2016). Antioxidants are also very beneficial for skin health, including protecting the skin from UV radiation which has the potential to damage skin cell tissue in humans, stopping premature aging, and protecting it from free radical molecules (Haerani, 2018).

Natural antioxidant from several plants needs to be researched in order to get the best antioxidant activity (Wicaksono, 2017). Natural plants that have been tested for their antioxidant activity are gotu kola herb and kelor leaf. Gotu kola herb extract contains triterpenoids, asiaticoside, asiatic acid, and madecassic acid. Gotu kola herb extract can prevent the body from free radicals, that is indicated by the IC_{50} value (Jatayu, 2018). Some research showed that Gotu kola extract has IC_{50} values of 481.64 ppm, 294.71 ppm, and 64.61 ppm (Wientarsih, 2013; Sumiati, 2019; Widyani, 2019). While Moringa leaf extract has IC_{50} values of 2151.33 g/mL,

103.98 ppm, and 89.305 ppm (Alamsyah, 2016; Tutik, 2018; Hasanah, 2017).

From the various studies, it is known that gotu kola herb and moringa leaves are known to have good antioxidant activity. The combination of the two materials is expected to increase the antioxidant activity because the two ingredients provide a synergetic effect. In this study, Gotu kola extract and moringa extract were combined with following ratio 1:1, 1:2, 2:1, 1:3 and 3:1 (Djoko, 2021). The antioxidant activity of the gotu kola and moringa extracts was determined using DPPH method.

METHODS

Equipment

The equipments in this research were Glass utility (Pyrex), Grinding (Getra), rotary evaporator (IKA Labortechnik), freeze dryer (New Brunswick), Oven (Memmert), ultraviolet-visible (UV-Vis) spectrophotometer (Dynamica), analytical scales (Kern).

Materials

The materials in this research were Ethanol (96%, Brataco), ethanol p.a (99%, Merck), DPPH (Sigma-Aldrich), and aqua dest (Smart Lab).

Plant material

Gotu kola were collected from Tawangmangu, Central Java, Indonesia and identified in Center for Research and Development of Medicinal Plants and Traditional Plants, Surakarta, Indonesia. Kelor leaves were collected from Blora, Indonesia and identified in Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

Extraction

Gotu kola herb and kelor leaves were macerated using ethanol 96% and the filtrate was concentrated using a rotary evaporator.

Phytochemical Screening (Endarini, L.H, 2016)

The alkaloid group was identified by adding 2N HCl and 9 mL aquadest to 0.3263g of Gotu kola herb extract, and 0.3597 grams of Kelor leaf extract then heated for 2 minutes before addition of mayer reagent. Presence of alkaloids in the sample marked with formation of a red precipitate.

The flavonoid group was identified by adding Mg powder and hydrochloric acid 2% of 0.3263 grams of Gotu kola herb extract, and 0.3597 grams of Kelor leaf extract. Presence of flavonoids in the sample marked with formation of a red-orange solution.

The saponin group was identified by adding hot water to 0.3263 grams of Gotu kola extract, and 0.3597 grams of Kelor leaf extract then cooled and shaked before addition of hydrochloric acid 2N. Presence of saponins in the sample marked with formation of a stable foam.

The tannin group was identified by adding aquadest to 0.3263 grams of Gotu kola herb extract, and 0.3597 grams of Kelor leaf extract then heated and cooled before addition of $FeCl_3$ 1% reagent. Presence of tannins in the sample marked with formation of a dark blue or blackish green solution.

The phenolic group was identified by adding sodium hydroxide to 0.3263 grams of gotu kola herb extract and 0.3597 grams of Kelor leaf extract. Presence of phenolics in the sample marked with formation of a red solution. Steroid and triterpenoid groups were identified by adding anhydrous acetic acid to 0.3263g of Gotu kola herb extract and 0.3597 grams of Kelor leaf extract then stirred gently till dried before addition of sulfuric acid. Presence of steroids in the sample marked with formation of a blue/green solution while the presence of triterpenoids in the sample marked with formation of a red or purple-red solution.

Antioxidant test by DPPH

The antioxidant activity of the extracts was determined using the DPPH free radical scavenging assay. Briefly, the universal bottle

was contained 10 µL of Gotu kola herb extract and Kelor leaf extract in concentrations from 0.08, 0.16, 0.24, 0.32 to 0.40 mg/mL and 5 mL 0.004% (w/v) solution of DPPH was added. The obtained mixture was vortexed, incubated for 30 min in room temperature in a relatively dark place and then was read using spectrophotometer at 516 nm. The blank was 80% (v/v) methanol. Ascorbic acid (Vitamin C) was used for comparison. Measurements were taken in triplicate. DPPH scavenging effect was calculated using the following equation 1.

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A}{A_0} \times 100\% \qquad (1)$$

where A_0 is the absorbance of negative control (0.004% DPPH solution) and A is the absorbance in presence of extract. The results were reported as IC_{50} values and ascorbic acid equivalents (ppm, mg/g) of Gotu kola herb extract and Kelor leaf extract.

RESULT AND DISCUSSION

Extraction

As shown in Table 1, the result of gotu kola herb extract was 163.14g and the yield was 16.31% from 1000 grams of simplicia. This result met the requirements of the herbal pharmacopoeia, where the requirement of the yield is not less than 7.3%. The result of Kelor leaf extract was 179.88 g and the yield was 17.99% from 1000 g. This result met the requirements of the herbal pharmacopoeia, where the requirement of the yield is not less than 9.2%.

Table 1. Results of Gotu Kola and Kelor leaf extracts

Sample	Yield (%)
Gotu kola herb extract	16,31
Kelor leaf extract	17,99

Phytochemical Screening

The phytochemical screening carried out on the ethanolic extract of Gotu kola herb and kelor leaf showed the presence of some bioactive compounds in the plant. Seven bioactive compounds of Gotu kola herb extract and kelor leaf extract were flavonoid, alkaloid, tannin, phenolic, saponin, steroid, and triterpenoid.

The results of phytochemical screening from Kelor leaf extract are in line with the results obtained by Luh Putu (2021). In the other hand, other study found the different bioactive compounds pattern (Yahya, 2020) where there is no triterpenoid content in gotu kola extract. This pattern can occur due to the differences in planting location, processing, climate, soil, and harvesting techniques which can affect the metabolites in Gotu kola herb.

Antioxidant Test by DPPH Method

Quantitative analysis of the antioxidant activity using the DPPH method was chosen because the test was simple, secure, fast, and sensitive. Measurement of the sample antioxidant activity was carried out at a wavelength of 516 nm. The presence of the antioxidant in the sample resulted in discoloration of the DPPH solution in methanol, which was initially concentrated purple to pale yellow. The antioxidant activity of the extracts of Gotu kola herb and Kelor leaves are expressed in the percent inhibition of DPPH radicals presented in Table 2. The best combination of Gotu kola herb extract and kelor leaf extract 2:1 was 56.65 ppm inhibition, which has a synergistic effect on free radical scavenging activity.

Table 2. Result of Antioxidant Activity test (IC_{50})

Samples	IC_{50} (ppm)
Gotu kola herb extract	76.66
Kelor leaf extract	82.66
Vitamin C	8.97
Gotu kola herb: kelor leaf (1:1)	74.07
Gotu kola herb: kelor leaf (1:2)	86.56
Gotu kola herb: kelor leaf (2:1)	56.65
Gotu kola herb: kelor leaf (1:3)	65.09
Gotu kola herb: kelor leaf (3:1)	67.58

CONCLUSIONS

The antioxidant activity test showed the best ratio for combination of gotu kola herb extract and kelor leaf extract was 2:1 which having a value of 56.65 ppm.

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