The effect of growing place on the oil quality and antioxidant activity of kelor (Moringa oleifera lamk) seed

N Ibrahim¹, S Nuryanti², A Hasanuddin³ and M S Zubair¹*

¹Pharmacy Department, Tadulako University, Palu, Indonesia
²Chemical Education Department, Tadulako University, Palu, Indonesia
³Department of Animal Husbandry, Tadulako University, Palu, Indonesia

Email*: sulaiman_zubair80@yahoo.co.id

Abstract. Kelor (Moringa oleifera Lamk) seed is part of the moringa plant that contains vegetable oil and has benefits as cholesterol-lowering agent, coronary heart risk, and cosmetic additives. It can be also used as edible oil and biodiesel oil. This study aims to determine the effect of different growing places on the oil quality and antioxidant activity of Kelor (Moringa oleifera L) seed. The plant was obtained from three different growing places, namely Palu City, Sigi and Parigi Moutong regencies. The determination of the oil quality refers to the Indonesian National Standard (SNI) that includes specific gravity, moisture content, acid value, iodine value, peroxide value, and saponification value. The results showed that Kelor seed oil from Sigi Regency had better quality than other places as indicated by the specific gravity, peroxide value, acid value and saponification value that fulfil the SNI 01-3742-2002 requirements. It also showed the lowest antioxidant activity with an IC₅₀ value of 2.93 mg/ml and the highest level of total phenolic and total flavonoids. This study encourages the application of Kelor seed oil from Sigi Regency as the best raw material for medicine and cosmetics.

1. Introduction
Kelor (Moringa oleifera Lamk) is a herbaceous plant that can easily grow in the tropical area, even in extreme condition. It is a well-known plant in Indonesia, particularly in Central Sulawesi, because the people used the leaves and seeds for daily vegetable soup. Several studies showed that M. oleifera seed has potency as antimicrobial, antitumor, anti-hyperuricemia, anti-inflammatory and antioxidant [1-4].

Standardization of extract of medicinal plants in Indonesia is one of the important stages in the development of Indonesian native herbal medicines. Medicinal plant extracts could be in the form of starting materials, intermediates, or materials for end-products. Extract as a starting material is analogous to a commodity of raw material for medicine that is processed into end-products by pharmaceutical technology. As a pharmaceutical product, it means that the extracts are packaged in capsules, tablets, pills, or in topical dosage forms [5]. The complete nutritional content and business potential of Moringa oleifera as a promising commodity have led researchers to further do phytochemical standardization. Therefore, in this study, Kelor seed that grows in Central Sulawesi has been evaluated for their oil quality and antioxidant activity using the 1,1-diphenyl-2-picrylhydrazil (DPPH) method and discussed the effect of different growing places.
2. Materials and methods

The material used was Kelor (*Moringa oleifera* L) seeds, aluminium chloride, Folin- Ciocalteu 50%, sodium carbonate, potassium acetate, 96% ethanol, chloroform, saturated potassium iodide, iodine, cyclohexane, starch 1%, 0.1 N sodium thiosulfate, 95% neutral alcohol, 0.1 N KOH, phenolphthalein (pp) indicator, KOH-ethanolic, 0.5 N HCl, acetic acid, potassium iodide 15%, and aquadest.

Fresh material of Kelor (*Moringa oleifera* L.) seeds from old plants were taken from three different growing places, namely the Palu city, Sigi and Parigi-Moutong regencies. The plants were then cleaned and weighed. The Kelor seeds were chopped into smaller pieces, then aerated in the open air protected from direct sunlight. After drying, dry sorting is carried out to separate samples that are in good condition. The samples were mashed in a blender to become a powder and weighed (dry weight of the sample). The obtained powder of Kelor seeds was then extracted by maceration using 96% ethanol solvent for 3x24 hours in a maceration jar. After that, the 96% liquid ethanol extract was then evaporated using a rotary vacuum evaporator until obtained viscous ethanol extract.

2.1. Determination of oil quality

2.1.1. Specific gravity. The specific gravity of the extract was determined as follow: 0.5 grams of extract was diluted in 10 ml of 96% ethanol (5%). Pycnometer was heated in oven at 105°C for 3 hours and calibrated by setting the pycnometer weight and water weight. The oil extract was put into a pycnometer then the excess of liquid extract was removed and weighed. The weight of the empty pycnometer is subtracted from the weight of the filled pycnometer. Specific gravity of liquid extract is obtained by dividing the weight of the extract by the weight of water [6].

\[
\text{Specific Gravity} = \frac{\text{Extract weight}}{\text{Water weight}}
\]  

(1)

2.1.2. Water content. An amount of 0.1 g of the extract was weighed in a calibrated cup and then dried at 105°C for ± 3 minutes using a moisture analyzer. The display was arranged auto and 0.0 min. The value appeared was recorded. The water content was calculated as follow [6]:

\[
\text{Water content (\%)} = \frac{\text{First weight} - \text{last weight}}{\text{Last weight}} \times 100\%
\]  

(2)

2.1.3. Peroxide value. The 0.5 g extract was added by 12 ml of chloroform and 18 ml of glacial acetic acid to the 250 ml flask. 0.5 ml of saturated KI solution and 30 ml of distilled water was then added and immediately titrated with 0.1 N Na₂S₂O₃ by using 0.5 ml of 1% starch as indicator until blue color disappear. The experiment was repeated three times. Peroxide value is expressed in mg-peroxide equivalent in every 100 g of sample [4].

\[
\text{Peroxide value (mg/100g)} = \frac{V \text{Na}_2\text{S}_2\text{O}_3 (\text{ml}) \times N \text{Na}_2\text{S}_2\text{O}_3 \times 1000}{\text{Sample Weight(g)}}
\]  

(3)

2.1.4. Acid value. Approximately 1.0 gram of sample (fat/oil) was added by 50 mL of neutral 95% alcohol. After being covered with a cooling back, heat it to a boil and shake it vigorously to dissolve the free fatty acids. After chilling, the fat solution was titrated with a standard solution of 0.1 N KOH using the phenolphthalein (pp) indicator, forming a bright red color that did not disappear for 30 seconds [4].

\[
\text{Acid value (mg/g)} = \frac{V \text{KOH (ml)} \times N \text{KOH} \times \text{BM KOH}}{\text{Sample Weight(g)}}
\]  

(4)
2.1.5. Saponification value. Approximately 1.5 grams of the extract was added by 50 ml of KOH-ethanolic solution. After being closed with a cooling back, boil carefully for 30 minutes. After cooling, the solution was titrated by standard solution of 0.5 N HCl and phenolphthalein (pp) as indicator. To find out the excess of this KOH solution, titration of the blanko solution was done [4].

\[
\text{Saponification value} \left( \frac{mg}{g} \right) = \frac{V \ \text{KOH (ml)} \times N \ \text{KOH} \times BM \ \text{KOH}}{\text{Sample Weight (g)}}
\]  

(5)

2.1.6. Iodine value. Approximately 0.5 grams of the extract was added by 10 ml of cyclohexane and acetic acid (3: 2) solution and 25 ml of wjs solution and stored in a dark place for 30 minutes. After addition of 20 ml of 15% KI solution and 40 ml of distilled water, the solution was titrated with 0.1 N Na$_2$S$_2$O$_3$ until the solution showed pale color. 1% starch as indicator was then added (the color of the solution becomes dark blue) and titration was continued again until the color of the solution turns white and the cyclohexan layer becomes pink [4].

\[
\text{Iodine value} \left( \frac{mg}{g} \right) = \frac{Va – Vb \ (ml)}{N \ \text{KOH} \times 12.692} \times \text{Sample Weight (g)}
\]  

(6)

2.2. Determination of total flavonoid and total phenolic
Total flavonoid and total phenolic were analyzed using aluminium chloride and Folin–Ciocalteu reagents method, respectively according our previous study [7]. Briefly, 10 mg of quercetin standard was dissolved in ethanol 96% and diluted to obtain standard solution of 2, 4, 6, 8 and 10 μg/mL. While 10 mg sample was diluted by 10 ml ethanol 96%. Standard and sample solution (each 1 mL) was put in test tube and added 3 mL of ethanol 96%, 0.2 mL potassium acetate 1 M, 0.2 mL of aluminium chloride 10% and 5.6 mL of distilled water. After incubation for 10 min, the measurement was performed by using Cecil CE7410 UV-Vis spectrophotometer at 376 nm. For total phenolic, it is used gallic acid standard with concentration range from 5 to 125 mg/L. Sample solution was prepared (1 mg/mL). Standards and samples (1:1) were added by Folin–Ciocalteu 50% and distilled water (1:1) and incubated for 5 min. After adding 2 mL aqueous sodium carbonate solution (7.5%, w/v) and incubation (15 min) in the dark room, the measurement was done by using Cecil CE7410 UV-Vis spectrophotometer at 765 nm.

2.3. Determination of antioxidant activity
The radical scavenging activity of Kelor (Moringa oleifera L) seed ethanolic extracts against the DPPH radical was determined according to our previous study [7]. Briefly, 0.1 mM DPPH solution and methanol solution of M. oleifera seed extract (concentration series of 75 – 175 μg/mL) were mixed (1:1). After 30 min incubation, the measurement was done by using Cecil CE7410 UV-Vis spectrophotometer at 515 nm. Vitamin C (positive control) was prepared as the same procedure. The experiment was done in triplicate and % inhibition was calculated:

\[
\% \ \text{inhibition} = \frac{Abs.\ \text{blanko} – Abs.\ \text{sample}}{Abs.\ \text{blanko}} \times 100\%
\]  

(7)

The 50% inhibitory concentration (IC$_{50}$) was obtained by probit analysis.

3. Results and discussion
Kelor (Moringa oleifera L) seeds were obtained from Palu City, Sigi and Parigi Moutong regencies, Central Sulawes. These areas were chosen based on the different of their elevation. Palu city is categorized as a place with high elevation (150 m height above mean sea level), Sigi regency as medium elevation (70 m height amsl) and Parigi moutong regency as low elevation (15 m height amsl) [7]. The
seeds were extracted by maceration method using ethanol 96% for 3x24 hours. Ethanol was chosen based on the nontoxic property [8]. The percent yield of rendamen is presented in Table 1.

### Table 1. The rendamen percentage of ethanol extract of Kelor (M. oleifera L) seeds

| Growing places   | Sample weight (g) | Extract weight (g) | Rendamen (%) |
|------------------|-------------------|--------------------|--------------|
| Palu city        | 720               | 60.22              | 8.35         |
| Sigi             | 860               | 64.12              | 6.18         |
| Parigi Moutong   | 650               | 45.66              | 7.01         |

### Table 2. The oil quality of Kelor (M. oleifera L) seeds

| Parameters               | Palu city | Sigi  | Parigi Moutong | SNI 01-3742-2002 |
|--------------------------|-----------|-------|----------------|------------------|
| Water Content (%)        | 0.99      | 0.97  | 1.29           | 0.30             |
| Specific Gravity (g/ml)  | 0.82      | 0.82  | 0.81           | 0.9              |
| Peroxide Value (mgEq/100g) | 0.28  | 1.05  | 0.40           | Max 1            |
| Acid Value (mg/g)        | 104.06    | 65.45 | 88.59          | Max 600          |
| Saponification Value (mg/g) | 172.05 | 197.57 | 219.45         | 196-206          |
| Iodine Value (mg/g)      | 73.24     | 77.94 | 84.02          | Max 45-46        |

Standardization of oil quality is carried out by physicochemical method which includes water content, specific gravity, peroxide value, saponification value, acid value, and iodine value (Table 2). The determination of water content is very important which is closely related to the stability both in the processing and preservation of food stuffs. The water content of ethanol extract of Kelor (Moringa oleifera L) seeds from Palu was 0.99%, Sigi Regency was 0.97% and Parigi Regency was 1.29%. This water content was determined to maintain the quality of the extract. In addition to determining water content, it can also be used to determine the amount of other volatile substances in the extract. These results indicate that the ethanol extract of Kelor seeds is not in accordance with the SNI standard for cooking oil, which is a maximum of 0.30%. However, it is still in accordance with the limit for water content of the extract, which is a maximum of 30% to avoid the rapid growth of fungi in the extract [9].

Specific gravity is defined as the ratio of the density of a substance to the density of water. The value of the two substances was determined at the same temperature. The specific gravity of the extract can be calculated by using a pycnometer. The extract was diluted to 5% solution using ethanol as a solvent. The results obtained from the three different growing places have met the SNI quality standards. It describes the mass per volume unit to provide a boundary between the liquid extract and the viscous extract. Specific gravity is related to the purity of the extract and contamination [6]. The determination of the level of oil damage was performed by measuring the value of peroxides. Most of the oil damage is caused by oxidase and hydrolysis processes (enzymatic or nonenzymatic). At the first time of the oxidation process, peroxide compounds will be formed. Furthermore, ketones and aldehyde compounds have formed that cause the oil to smell and rancidity taste, which is a sign that the oil has spoiled. Three replications were carried out for all samples and it was found that all samples fulfil the SNI 01-3742-2002 standards.

The determination of the acid value is very important in relation to the quality of the oil because the acid value is used to measure the amount of free fatty acids in oil. The greater acid value the higher fatty acid content and the lower the quality. Free fatty acids contained in oil come from the hydrolysis process
or due to poor processing because the hydrolysis process can take place with the addition of acid and assisted by heat. The results of the determination of the acid value of the three samples indicated that the quality of the oil was still relatively in accordance with the SNI standard. The saponification value is expressed as the value of milligrams of KOH needed to lather 1 gram of fat or oil. The determination of the saponification value is carried out to determine the properties of oils and fats. This test can be used to differentiate a type of fat from other types. From table 2, it can be seen that the sample from Sigi Regency has a saponification value that fulfils the SNI standard, which is an average of 197.57 KOH/g. It means that the tested sample has good quality.

A high iodine value indicates a high unsaturation of an oil or fat. The high amount of absorbed iodine indicates the value of double bonds or unsaturated bonds. The iodine value is also useful as an indication of the shape of an oil or fat. Fats with high iodine value are usually in liquid form, while fats with low iodine value are usually in solid form. From Table 2, it showed that the iodine value of Kelor seeds from three different growing places showed high iodine values.

### Table 3. Total phenolic, total flavonoids and antioxidant activity of ethanolic extract of Kelor (M. oleifera L) seed

| Parameters                  | Palu       | Sigi        | Parigi Moutong |
|-----------------------------|------------|-------------|----------------|
| Total phenolic (mg GAE / mg) | 0.639 ± 0.211 | 2.246 ± 0.142 | 0.452 ± 0.042 |
| Total flavonoids (mg Q/g)   | 6.619 ± 0.733 | 8.217 ± 0.684 | 7.898 ± 0.617 |
| Antioxidant Activity, IC<sub>50</sub> (mg/ml) | 4.42 | 2.93 | 3.31 |

Further examination on antioxidant activity was performed. From Table 3, it is showed that Kelor (Moringa oleifera L) seeds from Sigi regency has the lower IC<sub>50</sub> (2.93 mg/ml) than other extracts. This result is in accordance with the high value of its total phenolic and total flavonoid. This study supports the application of ethanol extract of Kelor (Moringa oleifera L) seeds from Sigi regency as a good antioxidant agent. Oil from Kelor seeds can be also used as a cosmetic ingredient and good material for olive oil in food and non-food products such as biodiesel and cosmetics [8, 10]

### 4. Conclusion

Ethanol extract of Kelor (M. oleifera Lamk) seeds from Sigi regency has fulfil the SNI 01-3742-2002 requirements which indicated by the specific gravity, peroxide value, acid value and saponification value. It has also the lowest IC<sub>50</sub> antioxidant activity comparing to others. Its antioxidant activity was supported by the high value of total phenolic and total flavonoids.

### Acknowledgement

Thanks to the Department of Pharmacy, Tadulako University for supporting the facilities to perform this study.

### References

[1] Donli P O and Dauda H 2003 Pest Management Sci 59(9) 1060–1062
[2] Bharali R, Tabassum J and Azad M R 2003 Asian Pacific Journal of Cancer Prevention 4(2) 131–139
[3] Nurlina I, Siti N, Asriani H, Muhammad S Z 2020 Pharmacognosy Journal Accepted
[4] Ogbunugafor H A, Eneh F U, Ozumba A N, Igwos-Ezikpe M N, Okpuzor J, Igwilo I O, Adenekan S O and Onyekwelu O A 2011 Pakistan Journal of Nutrition 10(5) 409-414
[5] Kunle O F, Egharevba H O and Ahmadu P O 2012 *International Journal of Biodiversity and Conservation* 4(3) 101-112

[6] Ministry of Health Republic of Indonesia 2008 Jakarta

[7] Sulastri E, Zubair M S, Anas N I, Abidin S, Hardani R, Yuliandi R, Aliyah 2018 *Pharmacog J.* 10(6) 104-s108

[8] Sudaryanto, Herwanto T, Putri SL 2016 *Jurnal Teknotan* 10(2) 16 – 21

[9] Voigt R 1995 *Gajah Mada University Press* Yogyakarta

[10] Ndabigengesere A and Narasiah KS 1998 *Water research* 32(3) 781-791