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The Antioxidant Activity of Kelor (*Moringa oleifera* Lam.) Leaves Based on Drying Method

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Abstract

Food consumption consisting of high antioxidants could improve health conditions, and prevent cell damage caused by free radicals. *Moringa oleifera* Lam. comprised a lot of nutrition like essential vitamins, anti-inflammation, anti-aging, anti-bacteria, anti-diabetic, anti-hypertension, and antioxidants. The optimum antioxidant level depended on the thermal process such as drying. This research aimed to determine the effect of type and temperature of drying on the antioxidant of *M. oleifera* leaves. Nested with two factors, type (cabinet dryer and oven), and temperature (40 ^{o}C, 50 ^{o}C, and 60 ^{o}C) which replied three times were applied. The result indicated that antioxidant was decreased significantly with increasing drying temperature. The result showed that antioxidant, phenolic, and flavonoid content with cabinet dryer was higher than oven. The increasing temperatures tend to decrease flavonoid; it was proved with 60 ^{o}C was 0.54 mg $g^{-1.}$ ± 0.035 mg $g^{-1.}$ The best treatment was cabinet dryer 50 ^{o}C with the highest antioxidant 69.26 % ± 1.38 %, phenolic 1.17 mg g^{-1} ± 0.051 mg g^{-1}, and flavonoid 1.41 mg g^{-1} ± 0.168 mg g^{-1}.

Keywords: Flavonoid, Medicine plant, Phenolic,Radical prevention, Safe herbs.

1. Introduction

World Health Organization (WHO) admits herbal medicines as valuable and available resources for Primary Health Care. *M. oleifera* is a substantial food commodity, which has enormous attention as 'the tropics natural nutrition'. The leaves, fruit, flowers and immature pods commonly are used as a highly nutritive vegetable, and as the extracts, it is able to be effective antimicrobial (Özcan, 2020), particularly in India, Pakistan, Philippines, and several countries in Africa (Saini *et al.*, 2016; Suzauddula *et al.*, 2019).

M. oleifera is proven to have multi-system effects in the human body (Saini *et al.*, 2016); it becomes a famous herb in the community, but it is insufficient scientific evidence to explain the mechanism and validate its efficacy apparent uses. *M. oleifera* is rich in the simple sugar, rhamnose called glucosinolates and isothiocyanates (Suzauddula *et al.*, 2019). It also composed free radical inhibitor, like phenolic (phenolic acid, flavonoid, coumarin, quinone, tannin, and stilbenes), nitrogen (alkaloid, amine, B-alanine), vitamin, terpenoids (carotenoid), and another endogenous metabolites.

Previous studies proved that *M. oleifera* leaves contain β-carotene, vitamin C, protein, calcium and potassium that act as good natural antioxidants sources. Thus, it was able to increase the shelf-life of fat foods due to the presence of various types of antioxidant such as ascorbic acid, phenolic, flavonoids, and carotenoids (Suzauddula *et al.*, 2019). The high concentrations of ascorbic acid, oestrogenic and β-sitosterol, calcium, phosphorus, vitamins A, B and C, riboflavin, α-tocopherol, folic acid, nicotinic acid, pyridoxine, β- carotene, protein, and in particular essential amino acids (methionine, cysteine, tryptophan and lysine) present in *M. oleifera* leaves made it a virtually ideal dietary supplement (Saini *et al.*, 2016; Suzauddula *et al.*, 2019).

There were several factors that affect the decreasing of antioxidant activity, such as increasing temperature and duration (Issa and Abd-Aljabar, 2013; Jiang *et al.*, 2017), extreme pH, and storage intervals (Issa and Abd-Aljabar, 2013). Therefore, optimum of drying temperature for *M. oleifera* is important to analyse. Drying refers to a process of water removed and decreasing of herbs moisture content, which aimed to prevent microbial and enzymatic activity, consequently product preservation for extend shelf life (dos Reis *et al.*, 2015; Kamaruddin *et al*, 2020; Suwati *et al*, 2021). The weight and volume reduction of plant will give positive consequences for distribution and storage. Nowadays, consumers are more concerned about healthy lifestyle, the demands for natural and safe herbs are tend to increase. Nevertheless, it was very little known about the *M. oleifera* leaves phytochemical components based on different drying methods and temperature. Therefore, this research aimed to determine the effect of drying process (method and temperature) on antioxidant activity, total phenolic, flavonoids content, and colour of *M. oleifera* leaves.

2. Materials and Method

M. oleifera leaves was harvested from the Temas village, Batu City, East Java, Indonesia that resulted from

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directly picking of the tree, and treated on the same day. Drying equipment that was used consisted of cabinet dryer and oven. Supporting equipment used include blenders, desiccators, glassware, erlemenyer, digital scales, wrapping plastics, cups, filter paper, and sieves (100 mesh). Nested design was applied with factor consisted of type (cabinet dryer and oven) and temperature (40 ^{o}C, 50 ^{o}C, and 60 ^{o}C) of drying.

Firstly, fresh *M. oleifera* leaves was sorted, washed, dried for controlling raw material used sun drying (T= 38 ^{o}C about 72 h), siege, filtered used 100 mesh, then followed by parameter analysis. The parameters tested for raw materials were water content, ash content, protein, fat, fiber content, vitamin C, and antioxidants and colour followed AOAC International (Latimer, 2019).

Fresh Moringa leaves used were dark green, furthermore were washed using running water and separated from the stalks, then spread on a drying pan. The Moringa leaves were dried as treatment using a cabinet dryer and oven with a temperature of 40 ºC to 60 ºC for 24 h. Then powdered using a blender, sieved used 100 mesh, and followed by parameter analysis consisted antioxidants (DPPH), phenolic (Folin-Ciocalteu reagent which has been diluted with water (1:10 v/v) and 4 mL Na_2CO_3 1M), flavonoid, and colour. The data obtained was tested using ANOVA (Analysis of Variance) and DMRT.

2.1. Determination of Total Flavonoid Content

Total flavonoid content was determined by aluminium chloride colorimetric assay adapted from Sembiring *et al*. (2018) with modification. Quercetin in concentration (30, 40, 50, 60, 70, 80, 90, 100) µg mL^{-1} were prepared in 96 % ethanol. 50 µL of extracts (1 mg mL^{-1}) was added to 10 µL of 10 % the aluminium chloride, followed by 150 µL of 96 % ethanol. 10 µL of 1 M sodium acetate was blended to the mixture in a 96 well plate. All reagents were mixed and incubated for 40 min at room temperature protected from light. The absorbance was measured at 415 nm Spectrophotomètre.

2.2. Determination of Total Phenolic Content

The total phenolic content was based on the 96-well microplate Folin–Ciocalteu method adapted from Sembiring *et al*. (2018) with some modifications. A total of 25 µL of the diluted extract of *M. oleifera* was mixed with 100 µL of 1:4 diluted Folin–Ciocalteu reagent and shaken for 60 s in a flat-bottom 96-well microplate, then was left for 240 s and 75 µL of sodium carbonate solution (100 g L^{-1}) were added. The mixture was shaken at medium continuous speed for 1 min. After 2 h at room temperature, the absorbance was measured at 765 nm using Spectrophotomètre. Gallic acid dilutions (10 mg L^{-1} to 200 mg L^{-1}) were used as standards for calibration.

2.3. Determination of Antioxidant Activity

Test was conducted in a 96-well plate according to Zahratunnisa *et al.* (2017) with slight modification. 20 µL extracts solution in different concentrations (100 mg L^{-1}, 500 mg L^{-1}, 1 000 mg L^{-1}, 1 500 mg L^{-1}) and 180 µL of DPPH solution 0.147 mM were added to each well. After 30 min incubation at room temperature in dark room, absorbance was read at 517 nm using Spectrophotomètre. Methanol was used as blank.

3. Result and Discussion

3.1. Raw M. oleifera Properties

Analysis of raw materials included water content, ash content, protein, fat, fiber, vitamin C, and antioxidants (Table 1). It showed that there were differences between the results of analysis and literature. The water content of *M. oleifera* leaf flour was 6.96 % and higher than literature (6.64 %). It due to the drying process that causes water content in material to evaporate. During drying, there is movement of water along with volatile substances. The purpose of the drying process was to reduce the moisture content, as a result material becomes more durable, reducing the volume for convenience of storage. Losses incurred during the drying process are changes in physicochemical properties and decreasing in the material quality.

Table 1. *M.oleifera* Leaf Flour Properties

Parameter	Analysis	Literature
Water Content (%)	6.96	6.64
Ash Content (%)	9.13	11.67
Protein (%)	23.17	23.37
Fat (%)	6.94	6.74
Fiber (%)	3.09	3.67
Vitamin C (mg100 g^{-1})	13.58	17.3
Antioxidant (%)	28.2	20

Noted: literature (Kurniawati *et al*., 2018)

The ash content of *M. oleifera* leaf flour amounted to 9.13 %, and lower than literature 11.67 %. The increasing temperature of drying process, followed by increasing the ash content in the leaves. It is caused of water content in the leaves, which is evaporated become higher, as the result more minerals left in the material. The ash content described *M. oleifera* mineral levels, and it was potential of source essential element such as sulphur (S), magnesium (Mg), potassium (K), (Al Juhaimi *et al*., 2017). Protein content was 23.17 % and in accordance to Al Juhaimi *et al*. (2016) that *M. oleifera* leaves have a high crude protein content up to 25 %, also contain tannins, saponins, and alkaloids (Suzauddula *et al.,* 2019). While fat content were higher about 6.94 %. This caused by differences in variety, climate, soil fertility and harvest age (Suzauddula *et al.,* 2019). Vitamin C was 13.58 mg 100 g^{-1}, it is probably due to the effect of heat on the leaves when doing bleaching with hot water. Vitamin C is easily dissolved in water. The antioxidant levels of *M. oleifera* leaf flour amounted to 43.61 %, and higher than literature. This is close related to the high temperatures that can cause some antioxidant compounds damaged (Rababah *et al.,* 2015).

3.2. Antioxidant activity of dried M. oleifera leaves

The drying temperature gave very significant effect ($P \leq 1$ %) on antioxidants activity, and phenolic of *M. oleifera* leaves. DPPH assay is a simple, acceptable and most widely used technique to evaluate the radical scavenging potency of plant extracts (its absorption spectrum at 515 nm to 528 nm) when it accepts a free radical species (Chithiraikumar *et al.,* 2017). The factors of decreasing antioxidant activity were increasing

temperature, extreme pH, and storage (Issa and Abd-Aljabar, 2013). The antioxidant activity was high loss in oven drying than cabinet dryer. Intense thermal process also might cause significant loss in antioxidant (Jiang *et al*., 2017), it showed by the lowest antioxidant activity was 28.05 % ± 1.54 % at oven drying 60 ^{o}C (Table 2). There was found naturally in plants as well as deactivate enzymes and degrade phytochemicals (Teixeira *et al*., 2014). The decreasing of antioxidant activities has also been correlated to Maillard- type antioxidants declined generation and accumulation.

Several literature reported there were linear correlation of antioxidant with phenolic and flavonoid content. The decreasing antioxidant levels due to the drying process also showed in the papaya leaves commodity (*Carica papaya* L.), tomatoes (*Solanum lycopersicum* L.) and ginger (*Zingiber officinale* Roscoe.) this is caused by during the drying process, loss of macromolecules such as polyphenols occurred, which was associated with the temperature and the length of time (Annegowda *et al*., 2014 ; Gümüşay *et al.,* 2015; Yap *et al*., 2020). Research on spearmint leaves also showed a similar downward trend based on differences in drying methods (cabinet, freeze and oven dryer) which resulted in the deactivation of degradative enzymes such as polyphenol oxidase, so as to degrade phenolic compounds (Orphanides *et al.,* 2013; Wojdylo *et al*., 2019).

3.3. Phenolic content of dried M. oleifera leaves

Phenolic compounds are good electron donors that substituted with hydroxyl groups on the aromatic ring, which could directly contribute to antioxidant action (Aryal *et al.,* 2019). The phenolic compound of cabinet dryer was higher than oven treatments and tend to enhance with increasing temperature of drying (Table 2). It was shown by the highest phenolic compound was cabinet dryer with 60 ^{o}C treatments about 2.75 mg g^{-1} ± 0.046 mg g^{-1} This result was consistent with experiment in Mediterranean herbs, that drying at 40 ^{o}C rapidly inactive polyphenol oxidase which caused by enzymatic processes (Nistor *et al*., 2017; Rababah *et al*., 2015; Udomkun *et al*., 2015).

The drying process did not immediately deactivate degraded enzyme, it was able to degrade phenolic compounds before the sample was completely dry. The phenolic content might be responsible for the strength antioxidant activity. The increase also has been attributed to the improving phenylalanine ammonia-lyase activity (on mild heating). The key enzyme in the phenolic synthesis or to the increased extractability by solvents usage. In addition, the non-enzymatic inter-conversion between phenolic molecules and precursors of phenolic molecules availability might have contributed to increase in heating process.

3.4. Flavonoid content of dried M. oleifera leaves

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups (Panche *et al.,* 2016). There is not significant effect of drying method and temperature on flavonoid content of *M. oleifera* leaves. Increasing drying temperature declined the flavonoid enzyme activity, which proved by the lowest was Oven with 60 ^{o}C treatment with 0.54 mg g^{-1} ± 0.035 mg g^{-1}.

Drying might breakdown some phytochemicals (Wojdyło *et al*., 2016), which affected cell wall integrity and caused some flavonoids component migration. In addition, the loss in flavonoids may be due to breakdown or leakage by chemical reactions includes oxygen, enzymes and light during drying process.

Several studies reported a linear correlation of antioxidant with phenolic and flavonoid content. The research in *Rhus flexicaulis* Baker described that high antioxidant activity might be attributed to the high phenolic and flavonoid content (Abdel-Mawgoud *et al*., 2019).

Table 2. The effect of drying method and temperature to the *M. oleifera* leaves antioxidants, flavonoid and phenolic

Treatment	Antioxidant (%)	Phenolic (mg g^{-1})	Flavonoid (mg g^{-1})
Cabinet Dryer 40 ^{o}C	63.45 ± 1.43 c	1.92 ± 0.054 c	0.72 ± 0.071
Cabinet Dryer 50 ^{o}C	69.26 ± 1.38 c	1.17 ± 0.051 b	1.41 ± 0.168
Cabinet Dryer 60 ^{o}C	52.42 ± 2.51 b	2.75 ± 0.046 e	0.74 ± 0.056
Oven 60 ^{o}C	28.05 ± 1.54 a	2.56 ± 0.034 d	0.54± 0.035
Oven 50 ^{o}C	35.98 ± 1.36 a	1.03 ± 0.008 ab	1.82± 0.053
Oven 40 ^{o}C	46.51 ± 3.29 b	0.95 ± 0.029 a	1.31± 0.092

Note: The value followed by the same letter is not significantly different according to Duncan's Test α= 5 %

3.5. Colour

Colour is substantial choosing factor of product, mostly the acceptable of processed vegetables or fruits depend on attractive colour (dos Reis *et al*., 2015; Sigurdson *et al*., 2017). Based on Table 3, the decreasing level of colour in oven was higher than cabinet dryer method, while intense drying (high temperature) accelerated colour reduction. This result indicated that cabinet dryer with 40 ^{o}C resulted in lighter colour. The drying affected changes in brightening, yellow, and appearance (Samoticha *et al*., 2016). The drying method gave significant effect on lightness (L) ($P \leq 5$ %) of leaves. Colour changes could be caused by chlorophyll pigments were reduced as the result of photo-oxidation reaction in the cells. In addition, there is a competition between peroxidase enzyme and chlorophylls (Chatatikun and Chiabchalard, 2013; Ramirez *et al*., 2015 ; Vergara-Domínguez *et al.,* 2013), while the drying method and temperature gave very significant effect ($P \leq 1$ %) on a colour of leaves. The trend of (a) values decreased in cabinet dryer, but increased in oven treatment with enhancing temperature. The (b) values indicated the height-browning index, the drying temperature gave significant effect ($P \leq 5$ %) on b colour. The browning degree as well as temperature increased (Benlloch-Tinoco *et al*., 2015; Udomkun *et al*., 2015)

Table 3. The effect of drying type and temperature to the *M. oleifera* leaves colour

Treatment	L	a	b
Cabinet Dryer 40 ^{o}C	48.33±0.577a	6.13±0.058c	17.43±0.208c
Cabinet Dryer 50 ^{o}C	48.23±0.153a	6.33±0.058c	17±0.001bc
Cabinet Dryer 60 ^{o}C	48.20-±0.173a	5.8±0.001c	16.47±0.451bc
Oven 60 ^{o}C	47.10±0.173b	1.47±0.351a	16.2±0.300b
Oven 50 ^{o}C	46.20±0.173b	2.73±0.058b	16.3±0.173bc
Oven 40 ^{o}C	46.40±0.361b	1.87±0.058a	14.43±0.404a

Noted: The value followed by the same letter is not significantly different according to Duncan's Test α= 5 %, (L = lightness; a = red/ green; b = yellow/blue)

4. Conclusion

Based on the test results, it can be concluded that *M. oleifera* leaves antioxidant of cabinet dryer was higher than oven. The treatment of cabinet dryer and temperature 50 ^{o}C showed the highest antioxidant about 69.26 % ± 1.38 %. The increasing temperature causes phenolic compounds and flavonoids decrease significantly. The best treatment is 50 ^{o}C cabinet dryer with the highest antioxidant activity 69.26 % ± 1.38 %, 1.17 mg g^{-1} ± 0.051 mg g^{-1} phenolic, and 1.41 mg g^{-1} ± 0.168 mg g^{-1} flavonoid. This treatment also in the second position of lightness 48.23 ± 0.153, and green 6.33 ± 0.058.

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