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# Drying Temperature Modulates Antioxidant Activity of Bitter Melon (*Momordica charantia* L.)

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**Abstract**
Bitter melon (*Momordica charantia* L.) is recognized for its antioxidant-rich bioactive compounds, notably flavonoids and polyphenols. Thermal processing may compromise these compounds, affecting antioxidant capacity. This study evaluated the influence of drying temperature on the antioxidant activity of bitter melon fruit using the DPPH assay. Samples were dried at room temperature and 50 °C, with activity assessed by absorbance at 518 nm and $IC_{50}$ calculation. Results demonstrated higher antioxidant activity at room temperature ($IC_{50}$ = 20.631 µg/mL) than samples dried at 50 °C ($IC_{50}$ = 44.390 µg/mL). These findings highlight that elevated drying temperatures reduce the radical scavenging potential of *M. charantia*, emphasizing the role of processing conditions in maintaining its functional quality.

## I. Introduction

Free radicals are unstable and highly reactive molecules because they have one or more unpaired electrons in their outer orbit. To achieve stability, free radicals react with surrounding molecules to acquire an electron pair. This reaction occurs continuously in the body, and if left unchecked, it can cause various diseases such as premature aging, cancer, liver disorders, and other degenerative diseases. Due to their highly reactive nature, free radicals easily attack healthy cells in the body, so the body requires defense mechanisms to neutralize these free radicals (Artati *et al.*, 2024).

Antioxidants are compounds that can neutralize free radicals, but the human body's natural production of antioxidants is insufficient to counteract the increase in free radicals. Therefore, antioxidant intake from external sources, such as vitamins A, C, and E, is crucial. Furthermore, consuming foods containing chlorophyll, flavonoids, polyphenols, and carotenoids can also serve as external sources of antioxidants (Belgur, Indrawati and Duly, 2021).

Bitter melon *(Momordica charantia* L.) is a horticultural commodity that is widely consumed by the public even though it tastes bitter. This fruit is rich in nutrients such as vitamins, calcium, iron, phosphorus, protein and fat. Apart from being consumed as a food ingredient, bitter melon is also used as an alternative medicine for various diseases, including as a treatment for fever caused by malaria and to lower blood sugar levels (Syafiqah and Damanhuri, 2023).

Bitter melon has long been consumed as a daily food and is also believed to be a traditional medicine for various ailments. Bitter melon contains saponins, flavonoids, polyphenols, alkaloids, triterpenoids, momordicin, cucurbitacin glycosides, charantin, butyric acid, palmitic acid, linoleic acid, and stearic acid (Septiningsih, Sutanto and Indriani, 2017).

One of the crucial post-harvest stages that influences the quality of herbal medicines is drying. The drying process affects the chemical compounds and pharmacological effects of medicinal plants, particularly compounds that function as antioxidants. The phenolic and total flavonoid content of herbal medicines, which have antioxidant activity, can be affected by the drying method used (Luliana, Purwanti and Manihuruk, 2016).

The DPPH (2,2-diphenyl-1-picrylhydrazil) method is a simple, rapid, and sensitive technique for measuring antioxidant activity. The principle of this method is based on the ability of antioxidants to reduce DPPH free radicals, which is indicated by a decrease in the intensity of the purple color at a wavelength of 518 nm.

Based on this explanation, this study aims to examine the effect of varying drying temperatures on the antioxidant activity of bitter melon extract using the DPPH method. The results are expected to provide information on the optimal drying temperature for maintaining the bioactive content of bitter melon and support its use as a natural antioxidant source.

## II. Research Method

### II.1 Tools and Materials

The tools used in this study were stirring rods, porcelain cups, funnels, filter paper, volumetric flasks, micropipettes, microplates, microplate readers, rotary vacuum evaporators, horn spoons, Eppendorf tubes, analytical scales, glass jars, ultrasonic, vortex, vials, water baths.

The materials used were bitter melon (*Momordica charantia* L.) from Pinrang Regency, South Sulawesi Province, DPPH, quarsetin, 70% ethanol, 96% ethanol, distilled water, and aluminum foil.

### II.2 Procedure

#### II.2.1 Sample Preparation

Bitter melon fruit *(Momordica charantia* L.) that has been taken is wet sorted to reduce dirt and foreign materials carried in the sample. After being washed and dried, the fruit is then cut into thin slices and dried at room temperature, placed on baking paper at room temperature for 3 days and for bitter melon fruit at 50°C. Place in an oven at 50°C for 1 hour. Then, the dried herbs are ground into powder.

#### II.2.2 Sample Extraction

Each bitter melon fruit powder was weighed at 50 grams. Each weighed powder was then extracted using the ultrasonic method, then added with 500 mL of 70% ethanol solvent and placed in an ultrasonic at a temperature of 30°C for 60 minutes. The extract solution was filtered using filter paper, remacerated three times with the same solvent and treatment. The resulting dilute extract was evaporated to obtain a thick extract.

#### II.2.3 Preparation of Sample Extraction

***Preparation Of DPPH Solution***

A 50 ppm DPPH solution was made by weighing 2.5 mg of DPPH powder, then putting it into a 50 mL volumetric flask and then dissolving it in 96% ethanol to a volume of 50 mL.

The maximum wavelength measurement was carried out by measuring the DPPH solution that had been incubated for 30 minutes at a temperature of 37°C and measured at a wavelength of 350-650 nm.

***Preparation and Measurement of Quarcetin Reference Solution***

A 50 ppm stock solution was prepared by weighing 2.5 mg of quercetin and then dissolving it with 96% ethanol in a 50 mL volumetric flask, then diluting it in several concentration series, namely 1, 2, 3, 4, 5, and 6 ppm, made by pipetting each stock solution with 0.1; 0.2; 0.3; 0.4; 0.5 and 0.6 mL, then adding 96% ethanol to a volume of 5 mL.

The test was conducted by pipetting 0.2 mL of sample solutions of various concentrations into Eppendorf tubes and then adding 0.2 mL of 50 ppm DPPH to each. After that, it was homogenized using a vortex, then pipetted 0.2 mL each into a microplate and incubated at 37°C for 30 minutes, then the absorbance was measured at a wavelength of 518 nm.

***Preparation And Measurement of Antioxidant Activity of Bitter Melon Fruit Extract***

A 1000 ppm stock solution was prepared by weighing 10 mg of each bitter melon extract and dissolving it in 96% ethanol in a 10 mL volumetric flask and then homogenizing it. Next, dilutions were carried out in several concentration series, namely 10, 20, 30, 40, 50, and 60 ppm. Each stock solution was pipetted at 0.05; 0.1; 0.15; 0.2; 0.25 and 0.3 mL, then made up with 96% ethanol to a volume of 5 mL.

The test was conducted by pipetting 0.2 mL of sample solutions of various concentrations into Eppendorf tubes and then adding 0.2 mL of 50 ppm DPPH to each. After that, homogenized using a vortex, then pipetted 0.2 mL each into a microplate and incubated at 37°C for 30 minutes, then the absorbance was measured using a microplate reader at a wavelength of 518 nm.

***Data Analysis***

The amount of antioxidant activity can be calculated using the following Formula 1

$$\% \text{ inhibition} = \frac{\text{Abs blanko-Abs sample}}{\text{Abs blanko}} \text{ x } 100\% \quad (1)$$

The sample concentration and % inhibition obtained are plotted on the x and y axes in the linear regression equation are as following Formula 2.

$$y = bx + a \quad (2)$$

From the equation: y = a + bx, the $IC_{50}$ value can be calculated using the following Formula 3.

$$IC_{50} = \frac{50-b}{a} \quad (3)$$

Note: y = 50 (50% antioxidant inhibitor), x = $IC_{50}$ (a number that indicates the concentration of extract that can inhibit the oxidation process by 50%), a = slope, b = intercept

## III. Results and Discussion

The sample used in this study was the ethanol extract of bitter melon *(Momordica charantia* L.) obtained by ultrasonic-assisted extraction (UAE). The sample extract was used for antioxidant activity testing using the DPPH method. The drying process was carried out at various temperatures to determine the effect of drying temperature on the stability of active compounds that act as antioxidants in bitter melon (*Momordica charantia* L.). The extraction result can be seen in Table 1.

**Table 1**. Extraction yield and percentage immersion of ethanol extract of bitter melon dried at room temperature and temperature 50°C

| Sample | Dried | Sample Weight (Gram) | Extract Result (Gram) | Extract Rendamen (%) |
|---|---|---|---|---|
| Bitter melon | room temperature | 264,01 | 64,68 | 24,499 |
| | temperature 50°C | 224 | 72,12 | 32,196 |

**Table 2.** Maximum wavelength result of DPPH 50 ppm

| Sample | Maximum wavelength | Absorbent |
|---|---|---|
| DPPH 50 ppm | 518 nm | 0.630 |

**Table 3.** Calculation of % inhibition

| Sample | Concentration (ppm) | Absorbance (518) | % inhibition | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|
| Quercetin | 1 | 0.396 | 37,142 | 32,209 |
| | 2 | 0.375 | 40,476 | |
| | 3 | 0.325 | 48,412 | |
| | 4 | 0.279 | 55,714 | |
| | 5 | 0.245 | 61,111 | |
| | 6 | 0.203 | 67,777 | |
| Bitter melon fruit at room temperature | 10 | 0.348 | 44,761 | 20,631 |
| | 20 | 0.318 | 49,523 | |
| | 30 | 0.286 | 54,603 | |
| | 40 | 0.254 | 59,682 | |
| | 50 | 0.229 | 63,650 | |
| | 60 | 0.204 | 67,619 | |
| Bitter melon fruit at temperature 50°C | 10 | 0.544 | 13,650 | 44,390 |
| | 20 | 0.463 | 26,507 | |
| | 30 | 0.413 | 34,444 | |
| | 40 | 0.33 | 47,619 | |
| | 50 | 0.272 | 56,825 | |
| | 60 | 0.228 | 63,809 | |

In this study, bitter melon fruit was used who comes from Pinrang Regency, South Sulawesi Province, takes fresh and easy bitter melon fruit, then cleans it using running water and dries it by airing it and avoiding sunlight.

Bitter melon fruit was extracted using UAE (Ultrasonic Assisted Extraction), a method used because it yields higher yields and requires less time (Widyasanti, Nurlaily and Wulandari, 2018). The solvent used was 70% ethanol, a solvent used during the UAE (Ultrasonic Assisted Extraction) process because Ethanol is able to extract more active compounds than other organic solvents. Furthermore, 70% ethanol was chosen because flavonoid compounds are typically polar glycosides and therefore must be dissolved in polar solvents, and 70% ethanol is a polar solvent (Hasanah and Novian, 2020).

The DPPH method was used for testing because it is simple, fast, and easy to screen for radical scavenging activity for several compounds (Sakka and Muin, 2022). Sample absorbance was measured using a microplate reader at a wavelength of 518 nm (Table 2) with a volume of 0.2 mL for each sample and DPPH. The sample concentrations used were 10, 20, 30, 40, 50, and 60 ppm for all sample variations, and for the quercetin comparison used as a positive control, concentrations were 1, 2, 3, 4, 5, and 6 ppm.

Measurement of each sample was carried out by calculating the % inhibition and $IC_{50}$ of bitter melon (*Momordica charantia* L.) fruit extract (Table 3). Percent inhibition is the ability of a material to inhibit free radical activity related to the concentration of a sample, while $IC_{50}$ is one of the parameters used to interpret the results of DPPH testing, the lower the $IC_{50}$ value of a sample, the greater its antioxidant ability (Table 4) (Aminah *et al.*, 2016).

**Table 4.** Antioxidant strength level (Rikantara, Utami and Kasasiah, 2022)

| No | Category | Concentration (ppm) |
|---|---|---|
| 1 | Very strong | <50 |
| 2 | Strong | 50-100 |
| 3 | Currently | 100-250 |
| 4 | Weak | 250-500 |
| 5 | Not active | >500 |

The results obtained in the antioxidant activity test of bitter melon extract (*Momordica charantia* L.) are the $IC_{50}$ value of bitter melon fruit that has been dried at room temperature of 20.631 µg/mL which means it is included in the very strong category and for samples dried at a temperature of 50°C obtained an $IC_{50}$ value of 44.390 µg/mL which is included in the very strong category (Table 3). From the results of the tests that have been carried out, it can be concluded that the antioxidant activity of bitter melon fruit samples dried at room temperature has the potential for stronger antioxidant activity compared to bitter melon fruit samples dried at a temperature of 50°C because the $IC_{50}$ value obtained is lower.

This is consistent with research conducted by Marbun et al. (2020), which found that antioxidant activity in samples tended to decrease with increasing drying temperature. In another study, Salsabillah et al. (2025) stated that higher drying temperatures can cause damage or evaporation of secondary metabolites in the extract.

## IV. Conclusions

From the Results demonstrated higher antioxidant activity at room temperature ($IC_{50}$ = 20.631 µg/mL) than samples dried at 50 °C ($IC_{50}$ = 44.390 µg/mL). These findings highlight that elevated drying temperatures reduce the radical scavenging potential of bitter melon (*Momordica charantia* L.), emphasizing the role of processing conditions in maintaining its functional quality. Therefore, can be developed at more varied temperatures, for example below 20°C, so that the optimum drying temperature range can be determined to maintain antioxidant activity in bitter melon fruit (*Momordica charantia* L.).

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