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# Optimization of Drying and Extraction Process of *Pereskia Bleo* leaves in Determining Antioxidant Properties Utilizing Pareto ANOVA

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**Keywords:** *Pereskia Bleo*; Drying, Extraction; Phenolic; Pareto ANOVA.

**Abstract.** *Pereskia Bleo* is commonly used among the local traditional medicine practitioners to prevent or treat cancer by consuming the leaves either raw or taken as a concoction brewed from fresh leaves. However, more research, regulation and standardization are required before herbal medicines can be recommended as effective and safe therapies. The objectives of this study are to obtain the optimum drying parameter and to identify the concentrations of phenolic content expressed as gallic acid equivalents (GAEs) in *Pereskia bleo* leaves. Pareto ANOVA method was used to study the optimum condition and the total phenolic content via F-Test using three samples for variance and three-factor with replication. Taguchi ANOVA was performed using SN ratio with L9 orthogonal array to accommodate the three factor level. The drying time were tested for 3 hours, 4 hours and 5 hours. The result shows that drying time is the significant factor in drying process and followed by drying temperature and solvent volume used. The drying experiments were conducted at operating temperature of 35 °C, 40 °C and 45 °C. While the extraction were conducted at different volume using methanol of 150 ml, 180 ml and 200 ml. The standard curve of total phenolic content was based on the gallic acid compound. The total phenolic content in C2 was at the most suitable for being used as antioxidant for consumption in human body with drying time of 5 hours at 45 °C by using 180 ml of methanol for extraction of *P.bleo* leaves.

## Introduction

The usage of medicinal herbs nowadays has expanded globally and driven by communities as a source of beneficial phytochemical compounds. Medicinal herbs also are demonstrated to have antioxidant, anti-inflammatory, antibacterial, analgesic, and antitumor activities [1]. One of the most important constituents involve in cell defense system against free radical is antioxidant [2]. Dietary intake of medicinal herbs with high contents of antioxidant can prevent damage to numerous cellular components caused by oxidizing agent, often in the form of free radical form [1].

In Malaysia, *Pereskia bleo* or locally known as 'Jarum Tujuh Bilah (in Malay) and Cak Sing Cam (in Chinese) [3][4] is a leafy cactus and distributed from Mexico to Brazil. The leaves of *Pereskia bleo* is consumed by local ethnic in Malaysia either in a raw form or in a concoction after having them boiled in water [5]. This plant has been cultivated in Malaysia for decorative and medicinal purposes. The potential of *Pereskia bleo* to treat diabetes, hypertension, rheumatism, cancer-related diseases, inflammation, gastric pain, ulcers, and for revitalizing the body was unlocked by several previous studies [5]. Medicinal herbs can be dried in a number of ways. Drying method is by far the most widely used [6] for the purpose of preservation. Drying process also was employed to control product moisture contents, minimize chemical degradation reactions, preserve product structure and texture, control product density and porosity. Although it can inhibit microbial growth, the method and temperature used for drying may have considerable impact on the quality that includes chemical and biological activities [1] of the resulting medicinal plant materials.

Extraction process are widely employed for the isolation of bioactive compound substance from natural sources. Soxhlet extraction method was selected due to its simplicity in operation, relative safety and potential for upscaling to industrial plant level [7]. Methanol was used as the extraction solvent due to its polarity and the well-known ability to extract bioactive compounds.

## Materials and method

**Drying Process** Pereskia bleo were collected from Universiti Teknologi MARA, Shah Alam in the morning. 30g of P.bleo leaves were dried by using an oven modeled ULE (MEMMERT). The drying process were conducted at temperature of 35°C , 45°C and 50 °C for 3, 4 and 5 hours respectively.

**Plant Extract** Prior of using Soxhlet extractor, the dry samples were grounded into small particles and placed in a thimble and extracted with different solvent volumes which were 150 ml, 180 ml and 200 ml of methanol. The extraction using Soxhlet extractor was refluxed at 80°C for 4 hours. The methanol removed in a rotary evaporator at 60-70°C in the water bath at 200 rpm.

**Total Phenolic Content** BHA (butylated hydroxyanisole) was used as positive reference standard in the study. All the test extracts and BHA were prepared at concentration of 20 mg/ml in methanol as stock extracts. 0.02 ml of the extracts at different concentrations was mixed with 1.58 ml of distilled water. 0.1 ml of Folin-Ciocalteu's phenol reagent and then added to each test tube. After three minutes, 0.3 ml of saturated sodium carbonate (Na2CO3) solution was added to the mixture. The reaction mixtures were incubated at 40°C for 30 minutes. The absorbance was determined at wavelength of 765 nm by using a spectrometer. A calibration plot using Gallic acid with concentration ranging from 25 to 1000 mg/l was prepared to perform a standard graph of Absorbance (Abs) versus Concentration (µg/ml). Gallic acid stock solutions in volumes ranging from 0.005 to 0.2 ml were pipette out into test tubes.

**Moisture Content** Total moisture content in leaves of *Pereskia bleo* were calculated based on dry basis using the following formula.

$$\text{X (dry)} = \frac{M_T - M_d}{M_d} \tag{1}$$

where; X(dry) = fraction of the moisture content (dry basis), $M_T$ = mass of sample (g) at a time, t(x), $M_d$ = dry mass of sample (g)

**Pareto ANOVA Method** In this study, Pareto ANOVA was employed to analyze the drying period and extraction process on antioxidant properties of *Pereskia bleo*. ANOVA is a statistical modeling methods and procedures related to review the variance in a variable where it is broken down into components that will give a different variance for each variable. Pareto ANOVA is a simple method of ANOVA. It determines Pareto diagram in this study. It is calculated by taking the total signal-to-noise (SN) ratio obtained from the L9 orthogonal arrays. For each level of 0, 1 and 2, the difference for each factor was squared. Sum of squares of each level was increased and the percentage was calculated based on the difference of squares of each level to the total.

## Results and Discussion

**Amount of Total Phenolic Content** Total phenolic content was determined by comparing absorbance value of the samples with standard curve of gallic acid obtained.

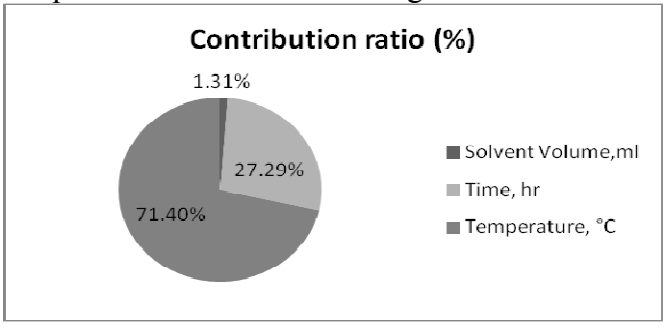


Figure 1: Contribution Ratio on each parameter to moisture content.

**Taguchi ANOVA (SN Ratio)** The initial mass of leaves affected to moisture content was recorded as the lowest, 1.31 % as indicated in Figure 1. The highest were 88.09 for B2 as shown in

Figure 2. Temperature of drying was the highest in contribution factor to moisture content which was 71.4 % due to *Pereskia bleo* leaves contained more water and humidity which is more than 60 % in Malaysia. In Figure 2, the sum of drying temperature, B2 was the highest (88.65 %) followed by 27.29 % of extraction time which affect the drying process and moisture content.

In drying process, the temperature and the duration of drying time were the most important factor affecting the process. In Malaysia, the humidity was at 60 % and the suitable drying time and temperature are needed to be identified in order to reduce the cost and time consumed. The solvent volume was not important to the drying process but to the extraction process as the highest percentage yield contributed to the total phenolic content and antioxidant property which was gallic acid. As conclusion in Taguchi ANOVA method to identify the optimum parameters and the combination factor was A1B2C0.

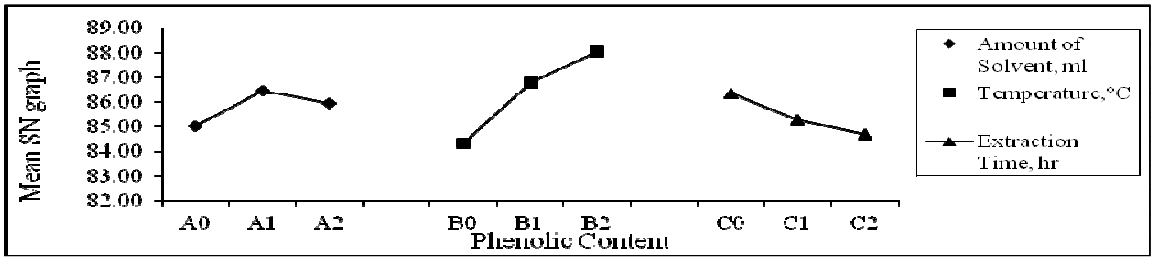


Figure 2: Mean SN graph versus moisture content.

Table 1: Summary result of moisture content (%) and total phenolic content (mg/l of GAEs).

| No of Run | Temperature of drying [$^0$C] | Extraction Time [hr] | Solvent Volume [ml] | Mass initial [$m_i$] | Mass final, [$m_f$] | Moisture content [%] |
|---|---|---|---|---|---|---|
| A0 | 35 | 3 | 150 | 30 | 26.9070 | 11.5 |
| A1 | 35 | 4 | 180 | 30 | 25.8035 | 16.3 |
| A2 | 35 | 5 | 200 | 30 | 25.7521 | 16.5 |
| B0 | 40 | 3 | 180 | 30 | 24.6498 | 21.7 |
| B1 | 40 | 4 | 200 | 30 | 26.8810 | 11.6 |
| B2 | 40 | 5 | 150 | 30 | 27.8293 | 7.8 |
| C0 | 45 | 3 | 200 | 30 | 24.7778 | 21.1 |
| C1 | 45 | 4 | 150 | 30 | 27.7008 | 8.3 |
| C2 | 45 | 5 | 180 | 30 | 28.8810 | 3.9 |

Table 2: Summary of Data for Phenolic Content (mg/l of GAEs)

| No of Experiment | Solvent volume, ml | Phenolic Content, [mg/l of GAEs] | Determination of safety |
|---|---|---|---|
| A0 | 150 | 112 | Unsafe |
| A1 | 150 | 117 | Unsafe |
| A2 | 150 | 120 | Unsafe |
| B0 | 200 | 133 | Unsafe |
| B1 | 200 | 125 | Unsafe |
| B2 | 200 | 121 | Unsafe |
| C0 | 180 | 115 | Unsafe |
| C1 | 180 | 113 | Unsafe |
| C2 | 180 | 106 | Safe |

**Three Factors with Replication** The three-factor with replication using solvent volume and the final mass of *Pereskia Bleo* leaves was done. The moisture content of the *Pereskia bleo* leaves for each experiment is shown in Table 1. C2 recorded the lowest moisture content of *Pereskia bleo* leaves after the drying process. The alpha value was assumed to be 0.05. Table 2 shows the summary of the Three-Factor with replication based on final mass (g) and solvent volume (ml).

Phenolic compounds are usually present in the form of plasma at concentration not exceeding 110 ppm during the consumption of fruits and herbs [8][9]. Hence, C2 shows the safe amount of total phenolic compound which is 106 ppm which is less of standard threshold level of total phenolic compound to be consumed.

Table 3: Summary of the three-factor with replication.

| Source of Variation | SS | dF | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Sample | 2501.93 | 3 | 833.98 | 448082.3 | $3.09 \times 10^{-21}$ | 4.066181 |
| Column | 86685.89 | 1 | 86685.89 | 46574909 | $2.38 \times 10^{-21}$ | 5.317665 |
| Interaction | 2498.11 | 3 | 832.70 | 447397.2 | $3.11 \times 10^{-21}$ | 4.066181 |
| Within | 0.01489 | 8 | 0.00186 | | | |
| Total | 91685.94 | 15 | | | | |

The P-value for the sample was $3.09 \times 10^{-21}$ and less than alpha value, 0.05 as shown in Table 3 and dependent to each other by mean final mass and solvent volume which affected the total phenolic content. The solvent play the main important roles towards the total phenolic content as the variance was higher than the final mass.

## Conclusion

In terms of the volume of solvent used to the total phenol content was 180 ml and less influenced by the weight of the final amount of herbs with total variance was less than the total volume of solvent. Methanol was often used as often because of the polarity was the highest compared to the other solvents. The total volume of methanol used is less since the antioxidant is soluble in methanol hence it could save the cost of getting antioxidant. The total phenolic content in C3 was at the most suitable to be used as antioxidant as total consumption of gallic acid was less than the other with drying time (5 hours), temperature of drying (45 °C) and volume of methanol (180ml). For energy efficiency ratio that was calculated, it was found that the fixed mass ratio, A1B2C0 factor was the optimum condition with only 4 hours to $35^0$C temperature and humidity in order to discover *Pereskia bleo* less than 10% of the original weight of 30g. The amount of methanol needed was 180 ml to obtain high concentration of antioxidants in the *Pereskia bleo's* leaves as this herb contains very high antioxidant content. Total phenolic content in this herb was influenced by the volume of solvent and less influenced by the amount of the final weight herbs. The total amount of solvents used such as methanol can be controlled to obtain the optimal value of total phenol content.

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