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Effect of drying temperature and age of leaves on total phenolic content on *Ficus deltoidea*

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Abstract. The objective of this research is to investigate the relationship between drying temperature and age of leaves on the total phenolic content of *Ficus deltoidea* leaves. The leaves were dried at 4 different temperatures which are 30, 40, 60 and 80 °C. The age of leaves is determined by using chlorophyll meter to measure the chlorophyll content and categorized into three level of age leaves. The total phenolic content of the *F. deltoidea* dried leaves were determined using Standard Folin–Ciocalteu (FC) analytical methodology with methanolic extracts of the samples. The result showed that the total phenolic content is highest at 40°C drying temperature and at medium-aged of leaves. It is suggested to consider drying the *F. deltoidea* leaves at 40-80°C and harvest at the medium-aged of leaves maturity to obtain high total phenolic content.

1. Introduction

Ficus deltoidea is locally known as *mas cotek* in Malaysia and it is one of the traditional alternatives and is still widely used among them [1]. Currently, *F. deltoidea* extract is being formulated into various of marketable products including pharmaceutical product and food supplement. It is proven that the bioactive compound in *F. deltoidea* exhibits therapeutic effects such as antioxidant [2], antidiabetic [3] and anti-inflammatory [4]. It was known that the leaves extracts of *F. deltoidea* contain high number of phenolic compounds with a good antioxidant activity [5].

Biologically active substances, especially phenolic compounds, composition, content and function in plants is affected by different factors such as harvest time, various external factors including cultivation, storage conditions, processing, climate and genetic background. There are a few factors that can affect the content of the bioactive compound specifically the total phenolic content. The age of the plant materials is one of the important factors that should be considered [6]. A few studies have found that different maturity stage of leaves show different content of total phenolic content and the contents differ according to species. Some mechanisms are highly dependable on the maturity of plant leaves [7]. In order to get the maximum total phenolic content from the herbal material, it is important to know which is the best leaves maturity where numerous of phenolic content accumulated before harvesting.

The use of high temperature in drying process of herbal material is generally expected to prevent herbal material to deteriorate due to low moisture content and microbiology reaction and subsequently a longer storage shelf life [8]. However, an extremely high temperature could degrade certain bioactive compounds and actually reducing the effectiveness of the herbal materials [9].



Even though there are many related studies in this field, there is a lack of scientific information on the changes of total phenolic content of this herbal raw material that are related to the leaves age and drying temperatures. Hence the aim of this study is to investigate the effect of different drying temperature and various age of leaves on its total phenolic content of *F. deltoidea*.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals used were as follows methanol (100%) purchased from Sigma Aldrich, Folin–Ciocalteu’s phenol reagent purchased from Merck, gallic acid (98%) purchased from Sigma Aldrich, anhydrous sodium carbonate (99%) purchased from HmBG Chemical. Absorbance was measured with SP-3000 Nano Optima UV–vis spectrophotometer.

2.2. Samples

The samples of *F. deltoidea* leaves were grown and collected at Institute of Sustainable Agrotechnology (INSAT) of Universiti Malaysia Perlis (UniMAP). The cultivation technique was in accordance with MARDI recommendation guide manual. The plant *F. deltoidea* leaves were chosen randomly based on their leaves age by using chlorophyll meter (Konica Minolta, SPAD-502plus) to measure the chlorophyll content of the leaves before harvesting. Three levels of leaves maturity were harvested categorized as in Table 1 below as young, medium and old. After harvesting, the leaves were cleaned before furthering into the next process.

Table 1. Categorization of leaves age.

Age of leaves	Chlorophyll content ($\mu\text{mol}/\text{m}^2$)
Young	10-20
Medium	30-40
Old	50-60

2.3. Drying

The leaves were divided into each age of leaves. About 5 g of leaves from each category were dried using laboratory oven at different temperature of 30, 40, 60 and 80 °C until the weight of leaves reached a constant weight which took 4-6 days to achieve. Each of the sample were triplicated for each temperature.

2.4. Extraction

The dried leaves were extracted using methanol as solvent. Before the extraction process, the leaves were ground by using conventional blender. 1 g of the ground leaves was then mixed with 100 ml of methanol and incubated in laboratory shaker for four hours with 150 rpm agitation. The samples were filtered using filter paper and the extracts were kept in air tight bottles and stored at -20 °C for further analysis.

2.5. Total phenolic content assay

Standard Folin–Ciocalteu (FC) analytical methodology as referred to Almalki [10] with slight modification were used for the quantification of total phenolic components in the organic solvent extracts. For the quantification, 0.2ml of diluted extract were mixed with 1.58ml of distilled water. After that, 0.2m of freshly prepared FC reagent was transferred to the conical flask and mixed thoroughly for three minutes for proper mixing. To that, 1.0ml freshly prepared of 20% sodium carbonate solutions were added and incubated at room temperature for 2 hours. The change in the colour was read at 760 nm using UV-vis spectrophotometer and using distilled water as a blank. Gallic acid was used as a

standard phenolic compound. The amount of total phenolic compound in the extract were determined as mg of Gallic Acid Equivalent (GAE) per mg dry weight. The phenolic content was calculated with standard curve equation and using the formula below:

$$TPC = \frac{GAE \times V}{M}$$

where GAE stands for Gallic acid equivalent, V is the total volume of sample and M is the weight of sample.

3. Results and discussion

Figure 1 showed the calibration curve for gallic acid at different concentration.

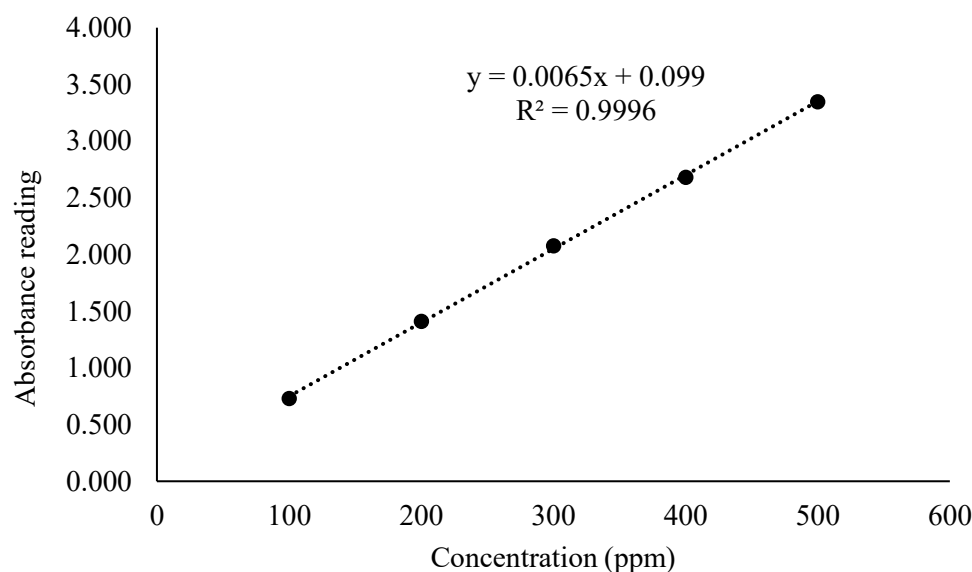


Figure 1: Calibration curve for Gallic acid at different concentration.

The data shows that the leaves age and drying temperature affected the changes of total phenolic content of dried *F. deltoidea* leaves. The total phenolic content of different leaves age and drying temperature yield between 17.229 to 63.883 mg/g. From this study, a trend can be seen where the medium-aged leaves showed highest value than the young and old leaves age. For the young leaves, the condition at drying temperature 60°C showed the highest total phenolic content. For the medium-aged leaves, temperature 30°C showed the lowest total phenolic content while the other temperature did not show any significant difference. For old leaves, the highest total phenolic content can be seen at when the leaves were dried at temperature 40°C.

Figure 2 shows that the medium-aged leaves reveal the highest total phenolic content compared with old and young leaves. The old and young leaves showed only little difference of total phenolic content. From the figure also, the total phenolic content of old leaves at drying temperature 40°C struck a higher value compared to young and older leaves. Therefore, the best age of leaves to gain the best total phenolic content is the medium-aged leaves.

The data in Figure 3 showed that the drying temperature gave significant effect to total phenolic content. Drying temperature 30°C showed the lowest total phenolic content. At drying temperature 40°C the antioxidant seemed to peak the highest when compared to the other drying temperature. However, at drying temperature 60°C, the total phenolic content seemed to slightly decrease and at drying

temperature 80°C the total phenolic content exhibited the lowest total phenolic content. This data showed that the total phenolic content for *F. deltoidea* are best obtained by drying the leaves at 40°C. On the other hand, the situation is different for tomato as that higher temperature the phenolic content of the species tend to directly proportional with the drying temperature [8,11].

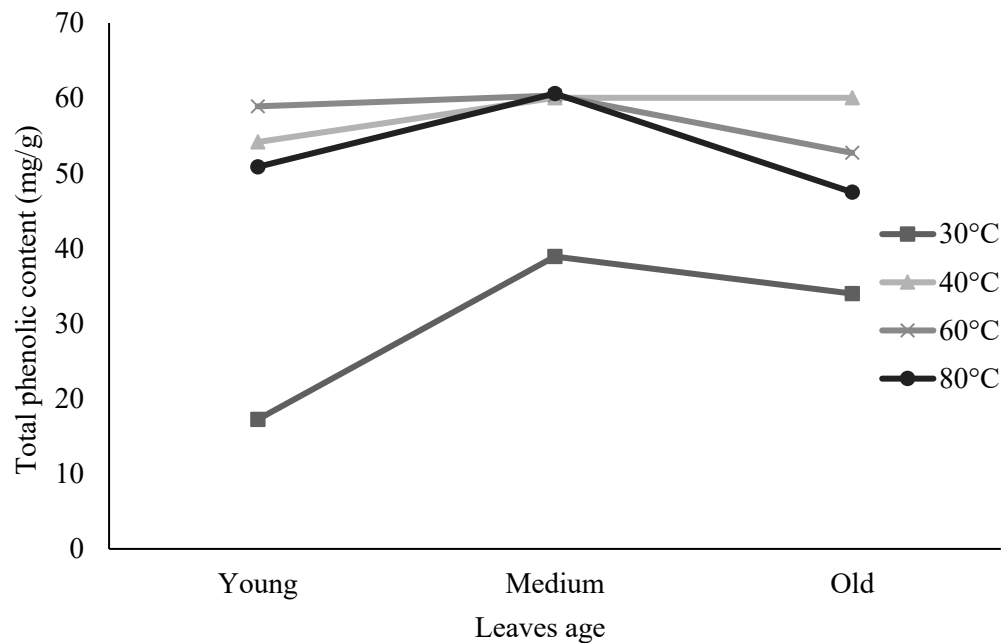


Figure 2. Total phenolic content of *F. deltoidea* leaves at different age of leaves.

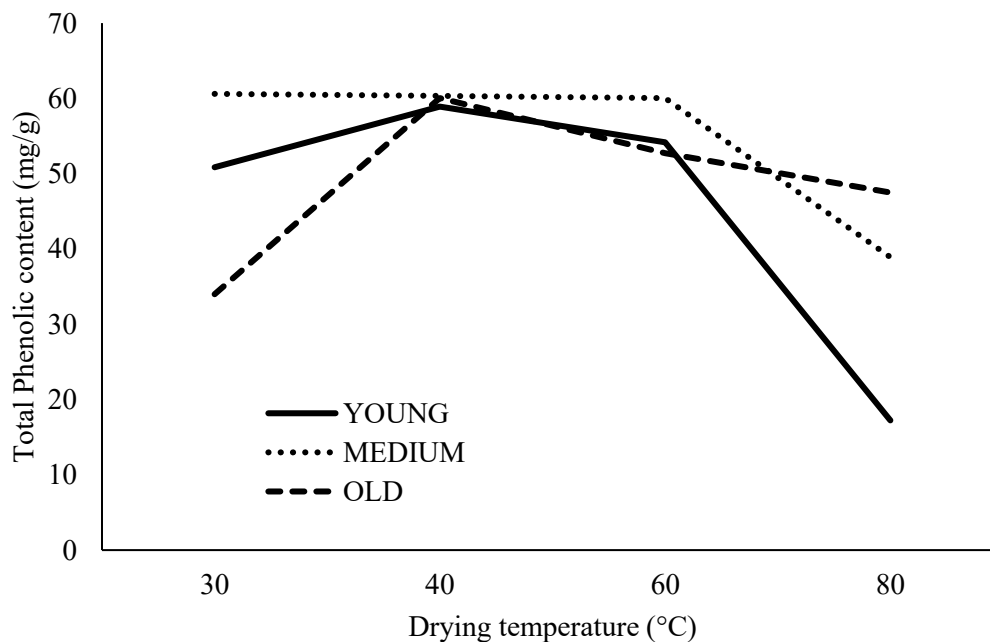


Figure 3. Total phenolic content of *F. deltoidea* leaves at different drying temperature.

Phenolic contents are generally different for each age of leaves. The old leaves tend to decrease in total phenolic content because of the maturity of leaves. The old leaves showed decrease in the production of phenolic compounds over time, since the contents are higher in leaves at young and medium-aged leaves. Thus, the age of the leaves has a significant influence in total phenolic content [12].

Medium-aged leaves have the optimum ability to produce secondary metabolites, so the levels of total phenolic content were large. For young leaves, secondary metabolites are still not produced in large quantities, while secondary metabolites content of the older leaves will gradually decrease. Besides this, seasonal, genetic, and agronomic elements can become external factors that can affect the level of total phenolics content [13].

4. Conclusions

As a conclusion, total phenolic content of *F. deltoidea* leaves are affected by drying temperatures and the age of leaves. The total phenolic content of this dried herbal materials is best obtained by harvesting medium-aged leaves and dried at temperature 40°C. The data from this study can also become an indicator for herbal practitioner and industry to increase the efficiency of *F. deltoidea* health products.

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