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The influence of leaf age and drying temperature on antioxidant capacity of *Cassia alata*

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Abstract. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics. *Cassia alata* plant has been commonly used as medicinal herbs for treating fungal infection such as ringworm and eczema. Nevertheless, there are only few studies focused on *C. alata*. This work aims to provide information about the antioxidant capacity of *C. alata* leaves at different stages of maturity and drying temperatures. The leaf maturity (young, medium and old) were determined by using chlorophyll meter. The leaves were dried at different temperatures (30, 40, 60 and 80 °C) using a laboratory oven. The leaves were then analysed for its antioxidant capacity. The extract of *C. alata* exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical. The antioxidant capacity in the examined extracts ranged from 85.81 to 89.71%. The results exhibited that the antioxidant capacity of *C. alata* extracts increased when the maturity of the leaves increased. Besides that, drying temperature was also found to increase the antioxidant capacity of *C. alata* leaves.

1. Introduction

In plants, leaf is one of the promising sources of antioxidants. An excellent classical example is tea leaves which have long been customarily consumed in China for more than 2,000 years [1]. Many factors are known to influence the amount and activity of leaf antioxidants such as genotype, season, sun- and shade acclimation and leaf age. In Malaysia, *C. alata* known as 'gelenggang', is among the prioritized Malaysian herbal plant species. This herbal species is known to have medicinal potential such as it was reported to be useful in treating skin fungal infection, convulsion, abdominal pains, oedema and is also used as a purgative [2]. Nowadays, many of *C. alata* products are available in the market in the form of tea sachets, pills and capsules. The remedial value in *C. alata* is due to its phytochemical compounds and antimicrobial activity [3]. However, there are few reports regarding the effect of leaf maturity on the metabolite content of the plant. During leaf maturation, changes in the oxidative metabolism of plant tissues occur [4, 5]. The accumulation and export of products also changes throughout leaf development. Therefore, the bioactive compounds of *C. alata* leaves collected at different stages of maturity (young, medium and old) were measured in this study.

Drying process is very important to extend the shelf life of a herbal product. Drying of herbs inhibits microbial growth and forestalls certain biochemical changes. However, at the same time it can also give rise to other alteration that affect herb quality, such as changes in appearance and the chemical properties of the products [6]. Nevertheless, drying variables, especially temperature and the drying method, can



alter the chemical constituents and appearance of herb quality. For example, the antioxidants and total phenolic content of *Garcinia mangostana* (mangosteen) was reported to decrease when dried at 70 °C compared to 60 and 50 °C [7]. Thus, the objective of this study was to investigate the effect of leaf maturity and also drying temperature on the total antioxidant activity of *C. alata*. This is important for determining on which leaf age is suitable and also to know the correct drying condition in obtaining the highest content of antioxidant capacity.

2. Materials and Methodology

2.1. Plant material

C. alata plants were grown at the Universiti Malaysia Perlis (UniMAP) Agrotechnology Research Station, Sg. Chuchuh, Perlis, Malaysia. The plants were grown from cuttings and maintained according to the standard practiced adopted by the majority growers in Malaysia.



Figure 1. Leaves of *C. alata* before separated and sorted into leaf age groups.

2.2. Sample preparation

The leaves were separated according to age, as defined by chlorophyll content measured using SPAD-502 chlorophyll meter (ranged from 0 to 50). The leaves were then removed from the twigs and proceeded to be washed and rinsed for further drying treatments.

Table 1. Leaf age grouping by chlorophyll content reading on SPAD meter.

Leaf age	Chlorophyll content reading
Young	10-20
Medium	25-35
Old	40-50

2.3. Drying process

About 10 g of *C. alata* leaves were dried by using four different temperatures (30, 40, 60, and 80 °C) by using laboratory oven until stable weight was achieved. The samples were replicated three times for each temperature and the data were recorded.

2.4. Preparation of extracts

The dried leaves of *C. alata* were extracted to determine its total antioxidant activity. The leaves were grinded by using an electric blender. 1 g of ground leaves were mixed with 100 ml of methanol solution. The extraction was later carried out in an orbital shaker at agitation speed of 150 rpm for four hours at the temperature of 40 °C. The extracts were filtered and the filtrates were kept in air tight bottles. The filtrates were refrigerated at -20 °C until further analysis.

2.5. Antioxidant activity determination

Antioxidant activity was measured in terms of reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The free radical (DPPH radical) scavenging activity was determined by the method of Shimada et al. [8] and Yang et al. [9].

The antiradical activity of the extracts were evaluated by using 2ml of DPPH and were added to 1 ml methanolic solution of extract in dark. The reaction mixture was allowed to stand for 1 hour in ambient temperature and the absorbance was measured at 517 nm against methanol as blank. Methanolic solution of DPPH was used as a control. All the samples were analysed in duplicate. The percentage of DPPH radical scavenging activity was calculated using the following equation (1):

$$\text{Percentage inhibition (\%)} = \left(1 - \frac{A_s}{A_c}\right) \times 100 \quad (1)$$

where, A_s is the absorbance of the control and A_c is the absorbance of the tested sample.

3. Results and discussion

3.1. Antioxidant capacity of *C. alata*

Figure 2 shows the results obtained for antioxidant capacity of *C. alata* leaves relative to age and drying temperature. The total antioxidant activity in the examined plant extracts using the DPPH solution. The antioxidant capacity in the examined extracts ranged from 85.81 to 89.71%. The highest total antioxidant activity was measured in old leaves dried at 80 °C while the lowest was measured from young leaves dried at 30 °C.

The results in Figure. 2 showed that the antioxidant capacity of the dried leaves increased as the maturity increased. The antioxidant activity of old leaves was higher than the young and medium leaves overall. Same trend was obtained from drying temperature on the antioxidant capacity of *C. alata* leaves. As drying temperature increased, the antioxidant capacity of the plant extract also increased. It has a constant increasing trend throughout the study.

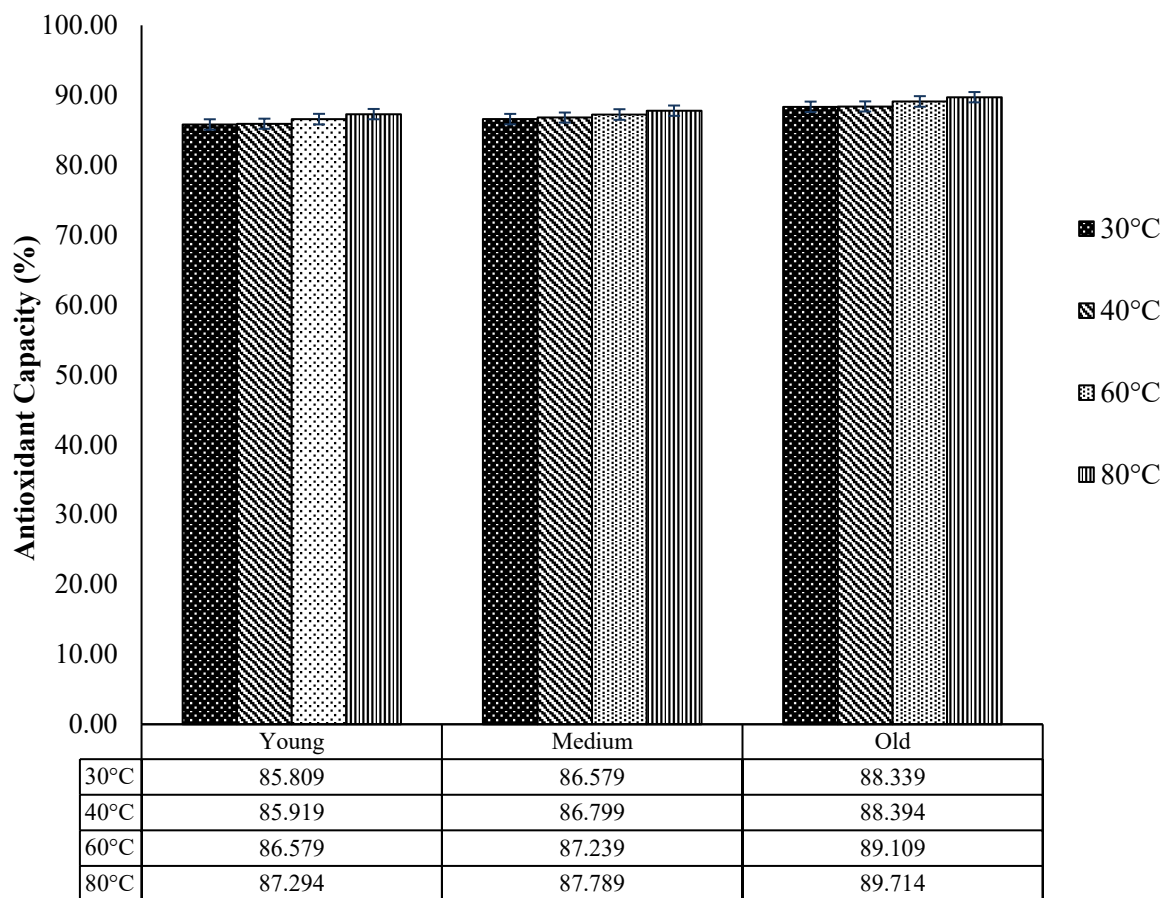


Figure 2. Effect of leaves' age on antioxidant capacity of *C. alata* at different drying temperatures.

3.2. Effect of leaves' age on total antioxidant activity of *C. alata*

The effects of drying fresh *C. alata* leaves at three stages of maturity with different temperatures were determined by using DPPH method. The highest amount of antioxidant activity was found in old leaves at 89.7% meanwhile the lowest was found in young leaves at 85.8% leaving with 3.9% of difference. Some of the studies done previously regarding age and antioxidant capacity also showed similar trend as the age increased, the total antioxidant activity increased. For instance, a research done on *Cassia fistula* showed a higher performance of antioxidant capacity as the leaves matured compared to younger leaves [10]. Another study done on *A. beccariana* leaves also observed the same trend as mature leaf extracts contained more antioxidant activity when compared to young leaves [11]. It was noted that there was an increased in production of antioxidant over time.

However, several studies showed different trend as the antioxidant activity decreased with age. For instance, a study done on aronia leaves showed the antioxidant capacity was higher in young leaves compared to old leaves [12]. Other study on yerba-mate and 'beauregard' sweetpotatoes similarly showed a higher total antioxidant activity at young leaves in comparison with old leaves [13,14]. Besides that, from the several studies done previously observed that there is a similar trend between the total phenolic compound and total antioxidant activity in regards to leaves age. Old leaves contain more phenolic compound and also antioxidant capacity. Furthermore, the antioxidant potential may be attributed to the presence of polyphenolic compounds [15].

3.3. Effect of drying temperature on total antioxidant activity of *C. alata* leaves

The result showed some slight increase in total antioxidant activity with increase in drying temperature. When *C. alata* leaves were dried at 40°C, the total antioxidant activity showed a small increase of 0.14% from 86.9% to 87.04%. The same pattern has been observed for temperature of 60 and 80°C as the total antioxidant activity insignificantly increased. A few studies have shown little or no changes in antioxidant levels after drying. This lack of alteration occurs because of the presence of heat-stable antioxidant compounds, such as carotenoids [16]. This is consistent with the experiment result which there is no significant change in total antioxidant activity content of *C. alata* leaves. However, there was also study found that drying generally causes the depletion of naturally occurring antioxidants due to the instability of these compounds induced by the heat of drying [17].

In addition, another factor to consider is the type of solvent used for extraction [18]. Methanolic extracts of fresh samples possessed both higher TPC and antioxidant activity than cool water extracts as methanol was able to denature polyphenol oxidases. Besides, being an organic and volatile solvent, it is more efficient in plant cell wall degradation, therefore, able to extract a greater amount of endocellular materials than water. Thus, this may also explain the increase and insignificant change of total antioxidant activity content in *C. alata* leaves compared to a constant decrease in total antioxidant activity when using water [19].

4. Conclusions

The effect of leaf maturity and drying temperature on antioxidant capacity of *C. alata* was investigated. From the data obtained in this study, it is shown that as the leaves matured, its antioxidant capacity increases. Furthermore, as drying temperature increased, total antioxidant activity of *C. alata* leaves also increased. This study suggests that the highest antioxidant capacity for *C. alata* was when the leaves are old and dried at 80 °C. High temperature by oven drying was the best in keeping antioxidant capacity of *C. alata* leaves.

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