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Abstract. *Centella asiatica* is one of the vital plant sources of antioxidants properties for consumption or medicinal purposes. This study aimed to determine the effect of three different drying methods (freeze drying, oven drying and dehydration drying) on the antioxidant of leaves of *C. asiatica*. The antioxidant properties and IC_{50} value of different concentration of methanolic extract solution (0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.062 mg/ml), were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The drying conditions and time required for freeze drying, oven drying and dehydration drying were -45°C for 3 days, 60°C for 5 hours and 35-43°C within 1-3 hours, respectively. Samples with lower IC_{50} value showed a stronger antioxidant activity since low IC50 determine the efec. The freeze-dried of *C. asiatica* sample at 0.05 mg/ml concentration exhibited the highest scavenging activity (93.97 ± 0.45%) with the lowest IC_{50} value (0.05 mg/ml) whereas the lowest scavenging activity was shown by dehydration dried of *C. asiatica* sample at 0.05 mg/ml concentration (55.08 ± 0.03%) with the highest IC_{50} value (0.36 mg/ml). This indicates that as the concentration increases, the radical scavenging activity increases. Thus, it is highly recommended to use freeze drying as the method to conserve the maximum amount of antioxidant properties in *C. asiatica* leaves.

1. Introduction

Centella asiatica (L.) Urb. (*C. asiatica*) has brought attention to substantial research and commercial interest due to the large number of bioactive compounds that promote health, especially phenolic compounds and triterpene saponins, which have several functional capabilities, including antioxidant activity and antimicrobial activity [1]. *C. asiatica*, commonly known as *pegaga* (Malaysia) or pennywort /gotu kola (English), can be found growing in the tropical regions of Asia, Oceania, Africa, and America [2]. *C. asiatica* is utilised as a culinary vegetable and medicinal herb. In Malaysia and Indonesia, although it has been used by traditional practitioners in their herbal medicines, it is commonly consumed as vegetable (*ulam* and salad) among the Malay and Javanese community instead of as a medicine [3]. High levels of antioxidants in this plant can be one of the potential alternative sources of natural antioxidant [3]. Recent studies also revealed the role of *C. asiatica* as an antioxidant, reducing the effect of oxidative stress *in vivo* and *in vitro* [4]. Higher DPPH-radical scavenging activity of 50% ethanol extract of *C. asiatica* compared to 100% ethanol and water

extracts [5] also proved that high antioxidant potentials of *C. asiatica* as a source of natural antioxidant [6, 7].

Drying of *C. asiatica* is one approach to obtain desired qualities, lessen storage space usage and prolong shelf-life for preparing the material for following pharmaceutical processing [1]. Drying can be performed with traditional methods such as sun or shade drying or modern methods such as oven drying, microwave drying or freeze drying [8]. In modern approach, aqueous extraction can be turned out into dried extract by adopting spray drying technique [9]. The presence of phytochemicals and health benefits [10] has increased the use of *C. asiatica* in food and beverages for many years [11]. The most similar study on the effect of different drying methods on flavonoid degradation in *C. asiatica* revealed that the freeze dried treatment gave the lowest total flavonoid degradation effect followed by vacuum oven treatment in the second place while air oven treatment caused the highest total flavonoid degradation [12]. Another research discovered that the dehydration drying can be done in various methods meanwhile the oven drying might be the easiest methods and quickest than the sun drying [8].

However, all these treatment methods might influence the antioxidant activity of *C. asiatica* plant specifically. Fresh leaves of *C. asiatica* has provenly contained high antioxidant properties, however due to short storage life of fresh leaves, the leaves need to be dried to prolong its shelf life and to prepare the material for subsequent pharmaceutical processing and commercial purposes or even for public use. Thus, the best drying method with minimal loss of antioxidant properties should be identified. Therefore, this research aimed to evaluate the antioxidant properties of *C. asiatica* leaves from three different drying methods namely dehydration drying, oven drying and freeze drying.

2. Materials and Methods

2.1. Plant materials

Fresh samples were obtained from a local market (Jeli District, Kelantan, Malaysia) and the species name was authenticated by botanist in Faculty of Agro Based Industry, Universiti Malaysia Kelantan. The best quality of samples was selected based on physical appearance such as young mature leaves. The leaves were washed with tap water three times to remove any residual soil and dust. The excessive moisture was removed by rinsing the plant leaves (together with the stems) with cool water, then they were shaken and pat dried with a paper towel. The samples were divided into three treatments (freeze drying, oven drying and dehydration drying), and the fresh weight for each treatment (70g) was measured for moisture content analysis.

2.2. Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (New Jersey, USA). Ascorbic acid and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). Only analytical grade reagents and chemicals were used in this study.

2.3. Drying methods

Three different drying methods; freeze drying, oven drying and dehydration drying were used to dry the samples (70 g each). Freeze drying method of *C. asiatica* leaves were applied according to the previous method [12]. The leaves were kept in ultra-low temperature freezer model MDF-U55V-PE (Panasonic, Osaka, Japan) for 24 hours. The samples were then placed in freeze dryer model CoolSafe 4-15 L (LaboGene, Lillerød, Denmark) for three days at -45 °C. A slightly modified method on temperature and time setting for drying the samples under oven drying was carried [8] as the samples were spread in the Forced Convection Oven Model OF-12G (Jeio Tech, Daejeon, Korea) and the temperature was set to 60 °C for five hours. For dehydration drying, samples were dried in the Arizona 6 Tray Food Dehydrator Model BCAZ6 (BioChef, New South Wales, Australia) following

the previous method [13]. The leaves were placed on the trays of the dehydrator in a single layer at 35-43 °C within 1-3 hours until the leaves were dried and easily crumbled.

The samples were dried until the constant weight of the samples were achieved. The moisture content of *C. asiatica* was determined after drying processes were completed. Dehydrator drying has the highest percentage of moisture content (89.34%) compared to oven drying (87.63%) whereas freeze drying contains the least moisture content which is 74.21%. After the moisture content was measured, the plant samples were ground into fine powder to obtain homogeneous samples [14] and to increase the contact of sample surface with the solvent system [15].

2.4. Methanolic extraction of samples

10 mg of dried sample was dissolved in 10 ml of methanol to prepare the extract solutions. These solutions were then sonicated for one hour. The filtrates were filtered using 90 mm SMITH Filter Papers to obtain the stock solution. Next, the stock solution was diluted with methanol at a ratio of 1:2.

2.5. Preparation of ascorbic acid standard

10 mg of ascorbic acid was dissolved in 10 ml of methanol (100%) to prepare a solution of ascorbic acid. This produced a stock solution with concentration of 10 mg/ml. The serial dilution was performed to prepare different concentrated solutions (0.5, 0.25, 0.0125, 0.062) mg/ml. The ascorbic acid solution (2 ml) of samples of various concentrations (0.5, 0.25, 0.0125, 0.062) mg/ml were placed in a test tube and 2 ml of methanol were added prior to DPPH scavenging activity evaluation test. Prior to DPPH scavenging activity evaluation test, 2 ml of methanol were added each into the solution of ascorbic acid (2 ml) of samples of various concentrations (0.5, 0.25, 0.0125, 0.062) mg / ml.

2.6. DPPH Radical Scavenging activity

A slightly modified method on concentration of samples was used for performing DPPH radical scavenging activity of extracts [14]. 4 mg of DPPH in 100 ml of methanol (100%) was dissolved in a dark place to prepare a solution of DPPH (0.004%). From this solution, 2 ml was taken each and added to a test tube which placed 1 ml of the methanolic solution of samples in a serial dilution (0.5, 0.25, 0.0125, 0.062) mg / ml. Without any disturbance, these solution mixtures were placed in the dark at room temperature for 30 minutes. Then, the samples were evaluated by reading the absorbance at 517 nm against a blank using a spectrophotometer. The indications in DPPH activity were observed with a colour change from violet to yellow. All these procedures were completed in triplicate with the use of ascorbic acid as the standard. The methanolic sample, standard ascorbic acid and DDPH solution were prepared on the same day of DPPH activity measurement. The following formula was used to calculate the percentage of scavenging activity on DPPH radical using the final absorbance reading of the sample:

$$Scavenging\ activity\ \% = (A_0 - \frac{A_1}{A_0} x\ 100 \qquad (1)$$

where A_0 is the absorbance control and A_1 is the absorbance sample. The result was reported as IC_{50} value. The IC_{50} value is the concentration of the sample which causing 50% of scavenging activity [15]. The IC_{50} values were calculated using linear regression equations. Lower IC_{50} value represents higher free radical activity [16].

2.7. Statistical analysis

All data was presented as the means of three measurements and error bars are displayed with standard deviation. The Statistical Package for the Social Sciences, IBM SPSS Statistics version 23 was applied to determine One-Way Analysis of Variance (ANOVA) with Tukey comparison test at 5% significance level ($p \leq 0.05$) within confidence interval of 95%. Microsoft excel with its Data Analysis add-in was used to determine the IC_{50} value from linear regression analysis.

3. Results and Discussion

The percentage of radical scavenging activity of different drying methods at different concentration; 0.5, 0.25, 0.125 and 0.062 mg/ml are shown in Figure 1. The results demonstrated that the DPPH free radical scavenging activity of the dried samples increased with the increasing concentration of the samples. From the results, the samples which undergoing freeze drying treatment displayed the highest antioxidant activities, followed by oven drying and dehydration drying treatment. Among all three drying methods, the freeze-drying treatment showed the significant highest radical scavenging activity ($p \leq 0.05$) in 0.5, 0.25, 0.125 and 0.062 mg/ml. In 0.5 mg/ml solution, the freeze-drying samples has the highest value of radical scavenging activity (93.97 ± 0.45%), leaving the standard ascorbic acid at the second place (90.58 ± 0.01), and followed by oven drying treatment (78.67 ± 0.44), while dehydration drying samples solution demonstrated the significant lowest ($p \leq 0.05$) radical scavenging activity (55.08 ± 0.03%).

However, oven drying exhibited the significant lowest ($p \leq 0.05$) percentage of radical scavenging activity at concentration of 0.25, 0.125 and 0.062 mg/ml which are 41.08 ± 0.31%, 40.43 ± 0.18% and 37.61 ± 0.26%, respectively. The dehydration drying treatment showed the second highest ($p \leq 0.05$) radical scavenging activity (46.50 ± 0.11, 41.81± 0.59, 38.44 ± 0.30) in 0.25, 0.125 and 0.062 mg/ml, respectively. As a standard antioxidant, the ascorbic acid proved to show positive control for the treatments since the scavenging activity recorded more than 50% in all concentrations even in the lowest concentration (0.062 mg/ml).

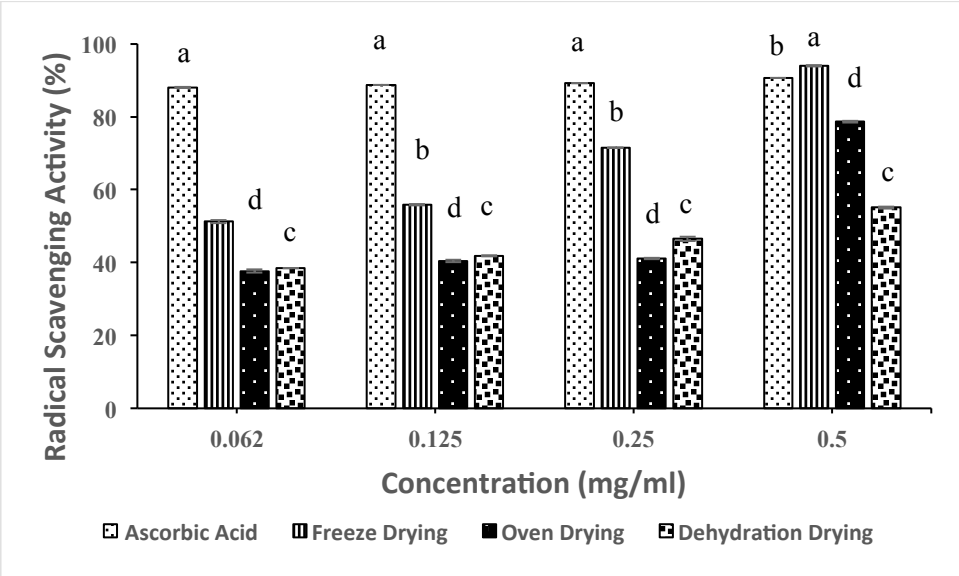

Figure 1. Percentage of radical scavenging activity of different drying methods and ascorbic acid at different concentration (mg/ml).

*Error bars represent standard deviation of the mean value of the three independent experiments.

Table 1 shows the results of IC_{50} value for each treatment compared to positive control (ascorbic acid). As for comparison, the higher the radical scavenging activity, the lower the IC_{50} recorded. The last column in the Table 1 displays the results of IC_{50} among all treatments. Since the ascorbic acid exhibited more than 50% of radical scavenging activity in all concentrations, thus it shows negative value of IC_{50}. It demonstrates that even though at lowest concentration of 0.062 mg/ml solution, more than 50% of inhibition of free radical activity recorded. The lowest IC_{50} represents the highest antioxidant activity (radical scavenging activity). From the results, the freeze-drying solutions gave

the lowest IC_{50} (0.05) among all three drying methods, followed by oven drying (0.24) and finally by dehydration drying (0.36). Hence, freeze drying proved to retain the highest free radical scavenging activity whereas dehydration drying retained the lowest. Hence, it can be proved that the freeze drying retained the uppermost antioxidant activity whereas dehydration drying retained the lowermost antioxidant activity.

Table 1: Percentage of radical scavenging activity and inhibitory concentration 50 (IC_{50}) value of *C. asiatica* of different drying methods at different concentration (mg/ml).

Treatment	Radical Scavenging Activity (%)				IC_{50} Value (mg/ml)
	0.5 (mg/ml)	0.25 (mg/ml)	0.125 (mg/ml)	0.062 (mg/ml)	
Ascorbic Acid	90.58 ± 0.01^{b}	89.19 ± 0.03^{a}	88.75 ± 0.03^{a}	88.09 ± 0.10^{a}	-7.02
Freeze Drying	93.97 ± 0.45^{a}	71.50 ± 0.17^{b}	55.81 ± 0.06^{b}	51.26 ± 0.23^{b}	0.05
Oven Drying	78.67 ± 0.44^{c}	41.08 ± 0.31^{d}	40.43 ± 0.18^{d}	37.61 ± 0.26^{d}	0.24
Dehydration Drying	55.08 ± 0.03^{d}	46.50 ± 0.11^{c}	41.81 ± 0.59^{c}	38.44 ± 0.30^{c}	0.36

$*^{a-c}$ Each value represented as mean ± SD (n=3). Means within different superscripts letter in the same column indicates significance difference ($p \leq 0.05$).

In antioxidant assay, comparisons were made based on the values of IC_{50}, which is the concentration of sample extract required to inhibit 50% of DPPH scavenging. The results are often expressed as IC50, which is the half the equivalent inhibitory concentration to give a 50% effect in scavenging the free radicals. Therefore, a lower IC_{50} value portrays a high radical scavenging activity in the samples [17]. Fifty percent of scavenging activity was determined from the curves plotted and the IC_{50} was obtained by linear regression equations. The highest radical scavenging activity in freeze-drying treatments was supported by the previous research which showed that freeze drying method gave the lowest IC_{50} value for freeze drying techniques of fermented local cocoa bean [17]. This is due to the degradation of bioactive compound [18] that might contribute to the antioxidant activity of *C. asiatica*. Another study also revealed that freeze dried samples have high outcomes in antioxidant estimation [19]. Freeze drying has high efficiency in removing moisture and retaining bioactive components, as well as antioxidant compounds in plants. The development of ice crystals within tissue matrix causing the tissue to become more brittle. Therefore, greater rupturing of cell structures results in higher solvent extraction of antioxidant compounds [19].

Comparing to fresh leaves, the drying absolutely reduced the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging capacity of the samples [1]. Measurement using Ferric reducing antioxidant power (FRAP) also showed that the increase in the drying temperature cause reduce of the antioxidant activity in the samples. From the methods mentioned in 2.3, the highest temperature was applied in oven drying (60ºC), followed by dehydration drying (35-43ºC) and the lowest temperature was freeze drying (-45ºC). From the principle, the low temperatures have been shown to contribute the high content of antioxidants in dry samples. Thus, the freeze drying treatment displayed the best drying method of *C. asiatica* with the highest antioxidant properties among the treatments.

4. Conclusion

Drying is a very useful technique in extending the shelf life of *C. asiatica* and to produce dried *C. asiatica* with high antioxidant properties. Drying methods were shown to exert significant effect on antioxidant properties. The higher the concentration, the higher the radical scavenging activity. Low IC_{50} values are preferred because they reflect greater antioxidant activity from the samples. Among the

drying methods tested, the highest antioxidant properties was demonstrated in the freeze drying treatment followed by oven drying and dehydration drying. For IC_{50} value, freeze drying has the lowest value (0.05 mg/ml) compared to oven drying (0.24 mg/ml) and dehydration drying (0.36 mg/ml). Thus, freeze drying is the most suitable method in displaying the highest amount of antioxidant properties in dried *C. asiatica*. Measuring the antioxidant properties of dried *C. asiatica* is beneficial for commercial purpose specially to maintain the shelf-life and product quality or at least lower the negative impact of heat towards antioxidant content. Therefore, those drying methods that preserve the highest level of antioxidant properties known for its health benefits will be of interest to the food and pharmaceutical industries.

Further research on antioxidant properties needs to be conducted using different part of plants such as leaves, stems and petiole and various type of species of *C. asiatica*. The method chosen for the analysis of antioxidant activity can be compared with the other methods such as using FRAP assay and 2,2'-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay method. This is to provide variation in the result and to find the most effective method in determining antioxidant properties. DPPH assay alone cannot be proven the antioxidant activity, thus more antioxidant assays can be conducted to strengthen the antioxidant claim in *C. asiatica.*

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