Changes in the physical and chemical properties of *C. nutans* herbal leaves dried under different drying methods

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Abstract. Drying is a well-known preservation method that uses to extent the shelf life of food materials during storage. In herbal industry, the drying is conducted by using several methods. It is believed that drying methods could affect the herbs physical and chemical properties. In this project, the effects of different drying methods on colour changes, rehydration ratio and bioactive constituent of *C. nutans* herb were investigated. The herb was dried under sunlight, shade, vacuum oven, and microwave. Results showed that the dried leaves have better retention of TPC and higher antioxidant activity as compared to the fresh leaves. Vacuum oven dried samples showed the highest antioxidant activity and significantly high in TPC. Besides, vacuum and under shade dried herbal leaves had no significant effect on the colour changes. Moreover, leaves dried under vacuum oven was also observed to exert the highest rehydration ratio due to less cell breakdown during drying. Therefore, vacuum drying has greater performance in term of retaining colour, less microstructure changes and greater TPC and antioxidant activity. It could be suggested that by adding vacuum to other drying methods may able to enhance the herbal quality.

1. Introduction
Nowadays, the use of medicinal herb healthcare products has increased rapidly over the years. Most of the people worldwide still relying on the natural based medicines for their primary healthcare. Since this practice continues, more new products being developed in the market. Hence, the intention on providing standard processing procedures including postharvest handling and downstream are needed to establish especially on Malaysia local herbs. As such, one of the locally well-known herbal plants *Clinacanthus nutans* (*C. nutans*) or also known as “Belalai gajah” or “Sabah snake grass” is used in this study. It is widely cultivated in Malaysia and its neighbouring countries. This herbal plant is commonly used as traditional medicine in South-East Asia region, particularly in Malaysia and Thailand for treatment of several illnesses such as skin rashes, insects and lesions caused by virus, diabetes, snakebite, and gout [1]. Apart from that, scientific studies have been carried out and proven that this herb exerts a few medicinal properties such as anti-herpes simplex virus [2], antiproliferative effects on human cancer lines [3] and antioxidant effects [4].
C. nutans herb is known to be highly susceptible towards microbial growth due to its high-water content. Hence, drying of C. nutans is crucial step as to reduce the risk of quality degradation. There are several methods used in the drying of herbal raw materials which include natural and artificial techniques. Despite disadvantages of natural sun and shade drying, the practices continue due relatively low cost and free energy. While artificial methods such as oven, freeze and microwave drying are able to overcome the disadvantages of using the natural method. Lower final moisture content, faster rates and more hygiene drying compartment are the advantages of using the artificial drying method. Studies have proven that the drying methods affect the herb final product quality. As reported in a research work on the drying of panelo, microwave-convective method was able to produce higher antioxidant content and total phenolic compound than the microwave dried samples [5]. Another research work conducted on peppermint leaves revealed that the drying by vacuum oven gave significant changes of the colour due to pigment degradation [6]. Moreover, earlier study has confirmed that drying could induce the formation of bioactive compounds mostly with antioxidant properties as a defence to biotic and abiotic stresses such as the rate of water reduction [7].

To date, a few studies on the drying of C. nutans herb were reported. For instance C. nutans leaves were dried by using air, oven and freeze dryer [8], solar dryer and heat pump assisted solar dryer [9,10]. The drying methods were found to give significant effects on the physical and chemicals properties of C. nutans. As reported by Khoo et al. (2015) air dried samples exerted higher total phenolic content (TPC) and antioxidant activity than that of the oven and freeze dry [8]. Ng et al., (2018) revealed that the heat pump assisted solar dryer gives better quality in term of higher extract yield, bioactive compounds orientin and vitexin, optimum colour and lower final moisture content [9]. However, the study on the effects of natural and artificial drying methods on physical and chemical properties of C. nutans herb is still lacking. Therefore, in this study the effects of sunlight, under shade, vacuum oven, and microwave drying methods on colour, rehydration ratio, antioxidant activity, and total phenolic content of C. nutans herbs leaves were compared. The information gathered in this study is important in order to provide standard post-harvest procedures of our local herb.

2. Materials and Method

2.1. Harvesting of C. nutans leaves

Fresh C. nutans herbal plants were harvested from Institute of Sustainable Agrotechnology (INSAT), Universiti Malaysia Perlis (UniMAP) production plot. The leaves were removed from the plant stems and rinsed with tap water to remove dust, soil and any dirt. Then the uniform size leaves were selected for experimentations purpose. The leaves were kept in air tight containers and placed in a refrigerator prior to use.

2.2. Drying experiment

C. nutans leaves were dried by using four different methods which were sunlight, under shade, vacuum oven and microwave. The leaves were dried until constant weight reached where the different between three consecutive readings recorded were less than 0.001 g. At this stage, the drying process was complete. The dried leaves samples were then kept in air tight containers to retained its moisture content and placed in a refrigerator prior to use.

2.2.1. Sun drying

An amout of 20 g C. nutans fresh leaves were weighed in triplicate. Then the leaves were distributed in a thin layer on flat perforated plates. The drying experiments was conducted under climate condition of UniCITI Alam Campus, Universiti Malaysia Perlis, Malaysia. The total drying process duration was about 17 hours between 11.30 am until 4.30 pm within 4 consecutive days. At other times, the samples were kept in an amber air tight glass bottle and placed in a refrigerator.
2.2.2. Under shade drying
An amount of 20 g C. nutans fresh leaves were weighed in triplicate. Then the leaves were distributed in a thin layer on flat perforated plates and placed in a room under ambient condition (at about 30°C). The samples weight were recorded every 2 hours until constant weight achieved. The drying experiment was conducted for 30 hours.

2.2.3. Microwave drying
An amount of 20 g C. nutans fresh leaves were weighed in triplicate. Then the leaves were distributed in a thin layer and placed on a glass rotating plate. A 1000 W microwave (Panasonic, NN-GD693SC) oven was used in this experiment. The weight of the sample was taken every 30 s until it became constant. The drying process completed in 8 minutes using fixed power level of six out of ten.

2.2.4. Vacuum oven drying
An amount of 20 g C. nutans fresh leaves were weighed in triplicate. The leaves were dried using a vacuum oven (DAIHAN Scientific, ThermoStable SOV-30) at a temperature of 60 ℃ under vacuum condition of 15 kPa. The samples weight were measured every 15 minutes until the drying process completed. It took 5 hours for completion.

2.3. Colour analysis
Colour changes of C. nutans leaves after drying were determined using colorimeter (KONICA MINOLTA Chroma Meter, CR-400). The color changes were evaluated using four factors of brightness (L*), chromaticity coordinate a* and b* and total colour change (ΔE). L* value was measured with a range from 0 to 100 which represents black to white. For chromaticity coordinate a*, it measures values from green (negative) to reddish (positive) colour while chromaticity coordinate b* measures yellow (positive) to blue (negative) [11]. ΔE was calculated by using the equation (1) below [12]:

\[ \Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \]  

(1)

where, \( L_0^* \), \( a_0^* \), and \( b_0^* \) were colour values for fresh leaves. Average value of five readings of \( L^* \), \( a^* \) and \( b^* \) were calculated.

2.4. Rehydration ratio
1 g dried C. nutans leaves samples were immersed in water bath at 35°C for 60 min. The weight of the rehydrated samples were measured after the 60 min soaking time. Excess water on the leaves surface was blotted with a tissue paper before weighing. The rehydration ratio (RR) of rehydrated samples was calculated by using equation (2).

\[ RR = \frac{W_r}{W_d} \]  

(2)

where, \( W_r \) = the weight of the leaves after rehydrated (g), and \( W_d \) = the weight of the dried leaves (g) before rehydrated.

2.5. Extraction of C. nutans leaves
The C. nutans leaves samples were extracted by using ultrasonic method. 1.0 g of fresh and dried leaves were ground with a laboratory blender. The ground leaves samples were mixed with 100 ml of distilled water and placed in an ultrasonic bath at a temperature of 40 °C. The leaves samples were extracted for 30 minutes, then filtered using a 11µm pore size filter paper (Whatman no. 1). The filtrate was collected and carefully refrigerated for further analysis of TPC and antioxidant activity.

2.5.1. Determination of total phenolic content in C. nutans leaves extracts
The extracts of *C. nutans* leaves were analysed on TPC according to Samarin et al. (2012) [13]. The extracts were added into 1 ml of 10-fold-diluted Folin-Ciocalteu (FC) reagent. Then, 0.8 ml of 7.5 % sodium carbonate was mixed and incubated under the dark condition for 30 min. After incubation the absorbance of the mixture was read at 765 nm by using a UV/Vis spectrophotometer with methanol as a blank. A standard curve was prepared with gallic acid as a standard.

2.5.2 *Determination of total antioxidant activity of C. nutans leaves extracts*

Total antioxidant activity of the leaves extract was determined by using free radical scavenging activity (FRSA) technique. 2 ml of 0.05 mg/ml methanolic solution of 1,1-diphenyl-2-picyrylhydrazyl (DPPH) were mixed with 200 µl extract and `methanol was added to make a final volume of 3 ml [14]. A mixture without leaves extract was used as control. After 60 min, the samples absorbance values were measured at 517 nm by using a UV/Vis spectrophotometer with methanol as a blank. The FRSA of the samples were calculated by using equation (3).

\[
FRSA = \left(\frac{A_c - A_s}{A_c}\right) \times 100
\]  

(3)

where \(A_c\)=absorbance value for control and \(A_s\) = absorbance value for the sample.

3. Results and Discussion

3.1. Effects of drying methods on the colour changes of *C. nutans* herbal leaves

The drying process is known to expose a substance under certain temperature, which gives an effect on the quality of the substance [15]. Colour is a quality attribute that significantly affects the consumer’s acceptability and determines the customer preference towards the food products [16]. Result on the effect of drying methods on the colour changes of *C. nutans* leaves is presented in Table 1.

| Sample                        | \(L^*\)         | \(a^*\)       | \(b^*\)       | \(\Delta E\) |
|-------------------------------|-----------------|--------------|--------------|-------------|
| Fresh leaves                  | 34.927 ± 0.9ad  | -10.793 ± 0.7a | 13.787 ± 1.1a | -           |
| Shade dried                   | 35.773 ± 0.7a   | -6.177 ± 0.6b | 12.613 ± 0.6ab| 4.838a      |
| Sun dried                     | 38.533 ± 0.6b   | -4.320 ± 0.3c | 14.930 ± 0.9b | 7.498b      |
| Microwave dried               | 30.650 ± 0.9c   | -5.783 ± 1.0b | 10.920 ± 1.2a | 7.184ab     |
| Vacuum oven dried             | 33.657 ± 0.7d   | -6.393 ± 0.4b | 12.657 ± 0.9ab| 4.717a      |

Means±SD within a column with different letters represent a significant effect (p<0.05).

As it can be seen from Table 1, the \(L^*\) values for sun, shade and vacuum oven dried leaves samples were higher than the fresh leaves. The higher \(L^*\) value is desirable in dried products [17]. In contrary, there was a significant reduction of the leaves green color (\(a^*\) value) after the drying treatments. This reduction happened due to chlorophyll degradation to pheophytins resulted from a loss of central magnesium ion [18]. Sun drying method showed the highest reduction of the leaves green colour as compared to the fresh leaves. The microwave, shade and vacuum oven dried samples did not show any significant difference in the \(a^*\) values and which were also higher than the \(a^*\) value of the fresh leaves. Similar finding was also reported in the drying of peppermint leaves [19].

The \(b^*\) value represents the blue (negative value) to yellow (positive value) color of the leaves. Result in Table 1 shows that the sun, shade and vacuum oven dried leaves presented the increase in yellowness color which were also due to the chlorophyll degradation [19].

The total color change of the fresh leaves after drying is represented by the \(\Delta E\) values (Equation 1). As shown in Table 1, sun drying method has highest total colour change towards the fresh leaves as it exerted a significant difference in \(L^*\), \(a^*\) and \(b^*\) values. However the other methods did not show any significant colour change as compared to the fresh leaves. In order to preserve the colour quality of the *C. nutans* leaves, sun drying would be the least desirable method to be used. Similar observation was also reported in the sun drying of green tea leaves [20].
3.2. Effect of drying methods on rehydration ratio (RR) of dried C. nutans leaves

Rehydration characteristics measure the induced damage of the material during drying. The ability of food products to reconstitute depends primarily on the internal structure of the dried parts and on the extent to which the water-holding components are formed [21]. Figure 1 illustrates the rehydration ratio of C. nutans leaves dried under different drying methods.

![Figure 1. Rehydration ratio of dried C. nutans leaves](image)

Among all drying methods, vacuum drying method gave the significantly highest RR value (2.982) followed by sun (2.765) microwave (2.703), and shade (2.665). Under vacuum condition, the leaves were exposed to relatively lower pressure than the other methods which lead to less cell breakdown. As higher RR value represents lower internal structure damaged of the dried leaves, drying under vacuum could be a preventive measure to avoid this damage. It may also cause by the enlargement or increment pores of the vacuum dried leaves. The porous structure is often associated with the increment of water adsorption ability [22].

Microwave drying also exhibits an expansion of macroscopic porous structure which results in better water retention [23]. However there is no significant different between rehydration ratio of sun dried and under shade dried samples. This may be caused by sunlight radiation that alters the structure of the cell wall which result in increment in rehydration ratio value as compared to shade drying. At ambient temperature condition during shade drying there was no adverse effect on the leaves cell structure resulted in lower RR [21].

3.3. Effect of drying on total phenolic compound and antioxidant activity

During drying some phytochemicals properties could degrade if the drying conditions are inappropriate. Most of bioactive compounds from plants are relatively instable which are very sensitive to drying treatments. Under certain conditions, indeed, drying may also help to protect these compounds and may not reduce their importance. In order to analyse the bioactive compound in C. nutans leaves, the Folin-Ciocalteau method was used to measure the total phenolic compound while antioxidant activity was determined by using DPPH free radical scavenging activity (FRSA). The results of total phenolic content and antioxidant activity for each drying method are shown in Figure 2.
As it can be seen from Figure 2 (a) and (b), *C. nutans* leaves dried under different drying methods showed a significant increase of TPC (1.94 – 2.16 mg GAE/g dry matter) and antioxidant activity (54.68 – 70.02 %) as compared to the fresh leaves (0.775 mg GAE/g dry matter and 38.9%, respectively). The increment of TPC and antioxidant activity of dried *C. nutans* leaves is because during drying process the cell wall of leaves was ruptured and allow the released of all bioactive compounds [24]. Similar observations were also reported on the drying of green tea leaves [25] and pigmented rice [26]. On the other hand, an increase of bioactive compound in the dried food material may also resulted from abiotic and biotic stresses response in plants. As reported in the previous work, rosmarinic acid, a phenolic compound in *O. staminues* leaves increased tremendously after drying.
[27]. It might be due the defensive properties of rosmarinic acid [28]. Earlier work by Hossain et al. (2010) also revealed similar observation [29].

By drying under shade, microwave and vacuum oven, the TPC contained in C. nutans leaves were not significantly different. Samples dried under direct sunlight also showed insignificant different with vacuum dried samples. Therefore, these drying methods would an option for the purpose of TPC enhancement. Further investigation could justiable in order to observe the preservation of specific phenolic compounds since it seems that the drying treatments tolerate the important bioactive constituent.

However, shade drying gave significantly higher antioxidant activity than the other methods. This may cause by during shade drying the samples did not expose to direct heat source. It may speculate that the antioxidant compounds constituent in C. nutans leaves is susceptible to direct heat. Similar observation was also reported in the previous work on the drying of O. staminues leaves [30]. Mediani et al. (2014) reported that shade drying resulting the highest values of TPC and antioxidant activity due to the slow loss of moisture content at ambient temperature without any external forces [31].

The antioxidant activity and TPC contained in C. nutans leaves were not comparable. It is because instead of phenolic type compounds, antioxidants also comprise of other types of chemical groups such as sugar. The samples dried under shade had the highest antioxidant activity significantly followed by vacuum oven, microwave and sun drying. Sudden moisture loss due short intense heat exposure time in the microwave drying may affect the antioxidant activity. Sun drying involves a direct sunlight treatment which the antioxidant may degrade due to harmful sun radiations. This is in accordance with the previous study of Orthosiphon stamineus leaves sun drying. The finding showed that the antioxidant activity in the sun dried samples was the lowest in comparison with shade and oven dried samples [30].

4. Conclusion
C. nutans leaves were successfully dried under sun, shade, vacuum oven, and microwave drying. The final product quality of the dried C. nutans leaves was observed based on both physical (colour and rehydration ratio) and chemical (TPC and antioxidant activity) properties. In terms of physical properties, vacuum oven method gave the better retention on colour (ΔE = 11.125) and high rehydration ratio (2.982) compared to the others. Dried leaves samples gave higher TPC and DPPH values than fresh leaves. Drying treatment improved the excretion of bioactive compounds during extraction and it may also increased by the plant response to biotic and abiotic stresses. A part from this, the TPC was insignificantly affected by the drying methods. Thus, any of the drying methods would be an option in order to preserve the TPC. Whereas shade drying gave the highest antioxidant activity significantly. As overall, vacuum drying is most preferable for the drying of C. nutans herb since it able to reduce the colour degradation, has high rehydration ratio, acceptable TPC content and antioxidant activity.

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