Effect of Drying Methods on the Colour Parameter, Rehydration Capacity and Antioxidant Activity of *Clinacanthus nutans* Leaves

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Proper drying method for natural product is crucial as it determines the physicochemical properties and antioxidant activity of the final product. In this work, drying methods were examined concerning their effect on the colour parameter, rehydration capacity and the antioxidant activity of *Clinacanthus nutans* leaves. The colour parameter and rehydration capacity were used as physical indicators of the quality of dried *C. nutans* leaves that were obtained. Three drying conditions were investigated, namely vacuum oven, oven (40-80°C), and air drying. It was found that vacuum oven drying of *C. nutans* leaves at 60°C resulted in the slightest colour change when compared to fresh sample. Vacuum oven drying method offers lower drying temperature that would be favourable to minimize degradation of thermally unstable bioactive compounds. Meanwhile, the highest rehydration ratio was obtained by oven-dried samples at 80°C followed by vacuum oven drying. Higher rehydration capacity is an indication that fewer damages occurred to the cellular structure of the product during drying. The extraction of *C. nutans* leaves were performed using supercritical fluid extraction in which air-dried sample obtained the highest antioxidant activity at 41.00 ± 3.07 mg TE/g. The results revealed that different drying methods significantly influenced ($p < 0.05$) colour parameters, rehydration capacity and antioxidant content of *C. nutans* leaves. The study also indicated that the colour parameter and rehydration capacity of samples did not represent the bioactive compound content of *C. nutans*.

Key words: *Clinacanthus nutans*; Sabah snake grass; *Belalai Gajah;* drying method; colour; rehydration*;* antioxidant

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Clinacanthus nutans (Burm. f.) Lindau is a local medicinal plant known as Sabah snake grass or *Belalai gajah* in Malaysia, *Phaya yo* or *Phaya plongtong* in Thailand and *e zui hua* in China [1-3]. *C. nutans* is a perennial herb widely distributed in Malaysia, Thailand and Indonesia [4]. It is a shrub with pubescent branches that can grow up to 1 meter in height [5]. Owing to the health benefits associated with *C. nutans*, the plants have been commercially propagated and sold in herbal markets as herbal teas and supplements [3]. As *C. nutans* is also consumed as herbal teas, the influence of different drying methods to the physicochemical properties of *C. nutans* leaves can also be used as benchmark for quality assessment of the final product.

The leaves are pale green in colour, with simple, narrowly elliptic, oblong or lanceolate-ovate shapes, that is estimated between 2.5 - 13 cm long and 0.5 - 1.5 cm wide (Figure 1) [2]. The stems of the plant are terete, striate and glabrescent while the petioles are sulcate, bifariously pubescent and between 0.3 cm - 2 cm long. The flowers formed in compact cymes that are dull red in colour with green base and yellow stripes on the lower lip [6]. The flower's upper lip is located in the throat and is triangular in shape with two stamens. The plant's ovary is compacted into two cells and each cell has two ovules [3]. Its capsule is oblong in shape and basally wrapped into a four-seeded short, solid stalk. *C. nutans* produce seeds that are about 2 mm in diameter [7].

C. nutans has been used as a traditional medicinal plant in Malaysia and other Asian countries to treat ailments such as nettle rash, fever, gout, dysentery, heals burns, blisters, insect bites, inflammation, urinates neuropathies, liver cancer, nasal cavity cancer, kidney problems and uterine fibroid [3-4,8]. In Thailand, the leaves are used as

Figure 1. Leaves and stem of *C. nutans.*

alternative medicine in the treatment of snake bite among traditional healers [9]. In Indonesia, the plant is used traditionally to treat dysentery, diabetes, and dysuria by consuming the decoction prepared by boiling the fresh leaves [10]. The plant is also used as anti-inflammatory agents in the treatment of allergic reactions and as natural remedies for herpes simplex [11].

The colour change that occurs in raw material during thermal processing is usually followed by colourimetry measurement as standard routine quality control [12]. Several studies have shown that the degree of colour change was dependent on the drying conditions of samples [13-15]. The statistical analysis of overall colour change ΔE in [13] showed that samples dried at 50°C obtained the lowest value, whereas the most significant colour loss was observed at 80°C. A study by [14] reported that drying process using low temperature such as 30°C and 35°C maintained the lightness of dried *H. hirsuta* L. leaves as compared to drying at 80°C. Another factor that can affect colour changes of the sample is the drying temperature. Higher drying temperature produced a greater colour changes that lead to darker colour [15]. From the colour parameter, the colour difference between samples and reference material were measured to evaluate the degree of colour changes that occurred during drying process.

Besides colour change, the rehydration ratio is also an essential characteristics of the quality evaluation of dry fruits and vegetables [16]. Rehydration ratio analyzed the capability of a dried sample to regain its original mass which is an indication of the damages in the cellular structure [17]. A correlation between the duration and severity of drying method with the speed and capacity of rehydration have been reported [18], which indicated faster and higher rehydration was obtained with decreased drying time.

The effect of different drying methods on bioactive compound yield is fairly significant as reported by various studies [14, 19-20]. A research by Pham *et al.* [14] reported that *Helicteres hirsuta* leaves dried under hot-air drying at 80°C and vacuum oven drying at 50°C obtained the highest total phenolic content (7.77 and 8.33 mg GAE/g, respectively) and total flavonoid content (5.79 and 4.62 mg CE/g, respectively), while leaves dried using infrared drying at 30°C had the lowest amount of bioactive compounds. A study by Anwar et al. [19] compared the total phenolic, and antioxidant activity of cauliflower (*Brassica oleracea* L.) extracts when using different drying processes, namely air drying, sun drying, and oven drying. The result indicated that as the length of drying time increased, antioxidant activity decreased since oven-dried samples obtained the highest crude extract yield, total phenolic content and antioxidant activity followed by the sun-dried and air-dried samples. Oven-dried cauliflower at 40°C for three days produced extracts high in antioxidants regardless of the extraction solvents used when compared to other drying methods [19]. Air-dried samples was reported to obtained higher phenolic content of *C. nutans* extract at 7.29mg GAE/g as compared to oven- and freeze- drying which resulted in 6.37 mg GAE/g and 5.10 mg GAE/mg, respectively [20].

The drying process acknowledged as one of the essential factors that can influence the quality of plant material. Among the changes in quality that can happen in any product during drying are the optical, sensory, and structural properties [15]. Drying is commonly used to preserved food in both traditional and industrial processing. Although drying inhibits microbial growth, it can also cause colour and aroma changes to the harvested plant due to degradation of bioactive compounds or the formation of new volatiles as a result of oxidation reactions or esterification reactions [21]. Researchers have

studied many drying methods on other medicinal plants such as sun drying, air drying, convection oven drying, freeze drying and microwave drying since it can lead to losses in the nutritional, physical and chemical composition of leaves [14-15, 22]. A recent study by Ng et al. [23] concluded that heat pump-assisted solar drying was more suitable than solar drying to obtain *C. nutans* samples with optimum colour values, low water activity and high flavonoid content. However, research on the drying behaviour of *C. nutans* leaves is still limited. The aim of this study is to determine the effect of different drying conditions on the quality of dried *C. nutans* leaves.

Materials and Methods

Fresh Material

C. nutans samples were collected from the herbal nursery at the project Plot-Demo UNDP/GEF-IDS located at Mile 30 Kimanis, Papar, Sabah, Malaysia. The plant was rinsed with tap water to remove any dirt and dried with tissue paper. The fresh leaves and stem were separated for further study.

Preparation of Dried *C. nutans* Samples

The fresh leaves of *C. nutans* were dried to constant weight using three different drying conditions as follows:

Vacuum oven drying. Three replicates of sample was vacuum oven dried according to the procedure described by Šumić et al. [24]. The leaves were spread on a glass petri dish and dried until constant weight in a vacuum oven (Model VO200 Memmert Vacuum Oven) at 60°C for 12 h with the pressure of 100 mbar (VO60).

Oven drying. Three replicates of each treatment was oven dried according to the procedure described by Fong [25] with slight modification. The leaves were spread on a glass petri dish and placed in the universal oven (Model UN110 Memmert Universal oven) at three pre-determined temperatures: 40°C (UO40), 60°C (UO60) and 80°C (UO80) until the samples achieved constant weight at 16 h, 6 h, and 3 h, respectively.

Air drying. Three replicates of the sample was air dried according to the procedure described by Khoo et al. [20]. Drying was carried out by spreading the leaves evenly on a tray, covered with cotton sheets to keep off dust and insects. The leaves were turned occasionally and left to dry in a shaded place with appropriate air flow for 2 weeks by which time they had turned brittle and dry (AD).

The dried *C. nutans* leaves samples were ground to fine powder with an electric blender (Model 7010S Waring Laboratory Blender) and then stored in a cold room (5°C) until used.

Preparation of *C. nutans* Extract

Supercritical fluid extraction (SFE) was performed using the Waters MV-10 ASFE system which comprises of extraction vessels, high-pressure pump, automated back pressure regulator, and oven. Liquid CO_2 was furnished from a gas cylinder. A total of 10.0 g of fine *C. nutans* powder was loaded into extraction vessel and the vessel is then placed in the oven. The extraction vessel was filled up with CO_2 to the pre-determined operating pressure and temperature for a total operating time of 120 min with CO_2 flowrate of 5 ml/min. The back pressure regulator maintains the condition to allow for efficient extraction. After the extraction time, the system was depressurized and the extract was deposited into the separator. The extract was filtered with Whatman filter paper No. 1, concentrated, evaporated, and stored in a refrigerator until further analysis. The conditions of the extraction process were set with reference to the study by Mustapa et al. [26], the pressure was set at 350 bar, temperature of 60°C, and extraction time up to 120 min.

Colour Measurement

The colours of fresh and dried *C. nutans* leaves samples were measured using chroma-meter (Konika Minolta CR400). The colour parameter, lightness (L*) value range from 0 (black) to 100 (white), redness (+a*), greenness (-a*), yellowness (+b*) and blueness (-b*) were recorded to study the changes in colour. In addition, the Chroma (C*) value and total colour difference (ΔE) were calculated using the following formula [27]:

$$\text{Chroma, } C^* = \sqrt{(a^{*2} + b^{*2})} \quad (1)$$

$$\text{Total colour difference, } \Delta E = [(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2]^{1/2} \quad (2)$$

Fresh *C. nutans* leaves were used as the reference, and a higher ΔE represented a greater colour change from the reference sample.

Rehydration Ratio

The rehydration tests of the dried sample were carried out by soaking the dried sample in distilled water at 100°C. A sample of 2.0 g was placed in a 250 ml beaker containing 150 ml distilled water. The beaker was covered and brought to a boiling point within 3 min and maintained at boiling for another 10 min. The sample was then filtered quantitatively using a Buchner funnel with Whatman No. 4 filter paper. The sample was then weighed. The rehydration ratio was calculated from the following equation [15];

$$RR = W_r / W_d \quad (3)$$

where, RR = rehydration ratio, W_r = drained weight of the rehydrated sample, and W_d = weight of dry

sample used for rehydration.

Determination of Antioxidant Activity

The DPPH assay was performed with slight modification according to the procedure described by [28]. Briefly, 0.1 ml of extract was added to 3 ml of diluted DPPH solution and incubated in the dark for 30 min so that the antioxidants present in the extract would react with the radical. The sample's absorbance was measured at 517 nm using a Genesys 10S UV-Vis spectrophotometer. The standard curve was prepared with Trolox and the results were expressed as mg TE/g extract. Three replicates of each sample extract were analyzed**.**

RESULTS AND DISCUSSION

Colour Values

Generally, the quality of tea sample is evaluated from the freshness, flavour, expected appearance, and texture of a sample [12]. Therefore, colour parameter analysis is a excellent indicator to for assessing the extent of colour change to surface appearance that transpires during the drying procedure. The *C. nutans* leaves were dried using three different drying conditions, namely vacuum oven at 60°C, oven drying (40°C, 60°C and 80°C) and air drying. The values of L*, a* and b* of fresh and dried *C. nutans* leaves were measured, and the C* and ΔE values were calculated (Table 1). Overall data showed that different drying treatments resulted in a significant difference ($p < 0.05$) between the colour parameters of *C. nutans* leaves (Table 1 and Figure 2). The fresh sample colour had the lowest L* value at 33.02, while all other dried samples had higher L* values. This indicated that sample colour increased in lightness when the sample was subjected to the drying process. Drying with lower temperature such as oven drying at 40°C and air drying obtained higher L* values as compared to other drying methods. Samples from air drying obtained the highest lightness value of 50.17, whereas those samples subjected to vacuum oven drying showed the lowest lightness value of 44.58. The fresh sample had a* value of -9.4 which indicates the green colour of the leaves. Oven drying at 40°C resulted in the highest a* value at -6.34, indicating that this drying method was able to preserve the green colour of the sample. The b* value obtained for fresh *C. nutans* leaves was 12.43 and all drying methods resulted in higher b* values which indicated that dried *C. nutans* leaves increased in yellow colour. The C* values of dried samples, which measure colour saturation or intensity, were in the range of 15.66 - 18.55. A more considerable ΔE value represented a more significant change in colour difference. The results from this study showed that ΔE from vacuum oven drying showed the lowest value when compared to fresh *C. nutans* leaves. In terms of quality, vacuum oven dried samples obtained the smallest change in colour differentiation when compared to the colour characteristic of fresh samples. This indicate that vacuum oven dried sample have the most similar in appearance to that of fresh samples. Vacuum oven drying conditions performed at lower drying temperature such as 60°C is favourable for samples containing bioactive compounds susceptible to thermal degradation. Most of the nutritional and sensory characteristics will be preserved since vacuum oven drying is carried out in the absence of oxygen at low temperature [29].

Table 1. Colour parameters of fresh and dried *C. nutans* leaves from different drying methods.

Drying Method*	Colour Parameter**				
	L*	a*	b*	C*	Δ E*
Fresh	33.02 ± 0.64^{a}	-9.4 ± 0.16^{a}	12.43 ± 0.35^{a}	15.58 ± 0.37^{a}	-
UO40	48.27 ± 0.37^{d}	-6.34 ± 0.11^{b}	$15.58 \pm 0.36^{b,c}$	16.82 ± 0.37^{b}	15.87 ± 0.29^{c}
UO60	46.78 ± 0.14^{c}	$- 4.97 \pm 0.07^{d}$	16.32 ± 0.25^{d}	17.06 ± 0.23^{b}	$14.97 \pm 0.62^{b,c}$
UO80	46.22 ± 0.50^{c}	-5.52 ± 0.11^{c}	$16.25 \pm 0.39^{c,d}$	17.16 ± 0.41^{b}	$14.29 \pm 0.65^{a,b}$
VO60	44.58 ± 0.63^{b}	-3.82 ± 0.09^{e}	15.19 ± 0.65^{b}	15.66 ± 0.65^{a}	13.15 ± 0.57^{a}
AD	50.17 ± 0.56^{e}	-4.88 ± 0.18^{d}	17.91 ± 0.18^{e}	18.55 ± 0.13^{c}	18.57 ± 1.01^{d}

* UO40/ UO60/ UO80: Universal oven drying at 40°C/ 60°C/80°C; VO60: Vacuum drying at 60°C; AD: Ai-drying in a shaded place with appropriate air flow.

** Data for colour parameter of *C. nutans* leaves are reported as means ± standard deviations of triplicate experiments. Those values in the same column with different superscript letters ($^{a-e}$) are significantly different ($p < 0.05$) according to Duncan's multiple range tests.

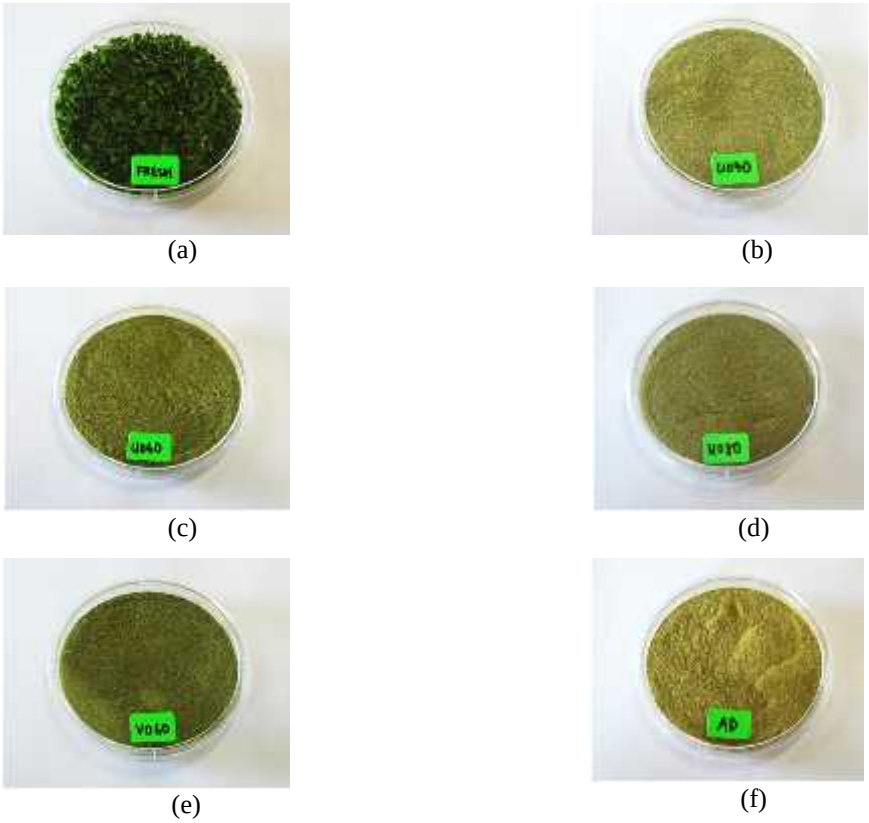

Figure 2. The colour characteristic of *C. nutans* leaves before and after subjected to different drying methods: (a) fresh leaves, (b) universal oven 40°C, (c) universal oven 60°C, (d) universal oven 80°C, (e) vacuum oven 60°C which obtained the lowest ΔE value, and (f) air-drying.

Rehydration Capacity

During the drying process, the shape and size of samples would go through changes due to shrinkage from moisture removal. The capability of dried material to regain its original mass is analyzed in terms of rehydration ratio, where a higher a value indicates better product that has less disruption in the cellular structure of plants [17]. The different drying treatments showed statistically significant effects ($p < 0.05$) on the rehydration characteristic of *C. nutans* leaves (Figure 3). The rehydration ratios ranged from 2.03 to 2.92. The lowest rehydration ratio was from air-dried samples followed by oven-dried samples at 40°C, in which both methods took longer drying duration as compared to other treatments. A similar finding was also reported by Moreira et al. [30] where samples dried over longer duration demonstrated lower rehydration ratios. Oven drying at 80°C resulted in the highest rehydration capacity followed by vacuum oven drying with values of 2.92 and 2.67, respectively. This may be due to faster moisture removal at a higher temperature which causes less shrinkage of dried samples [15]. The higher rehydration capacity obtained indicated that cellular structure damages were minimum in both drying process. In other words, higher values show better products that have less disruption in the cellular structure of plants, and therefore, the water absorption and the extent of restoration are higher [17].

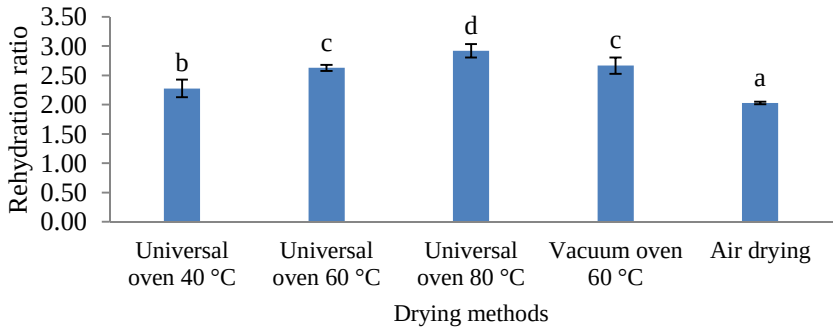

Figure 3. Effects of different drying methods on the rehydration ratio of *C. nutans* leaves. Data are reported as means ± standard deviations of triplicate experiments. Means with different letters (a-d) are significantly different ($p < 0.05$) according to Duncan's multiple range tests.

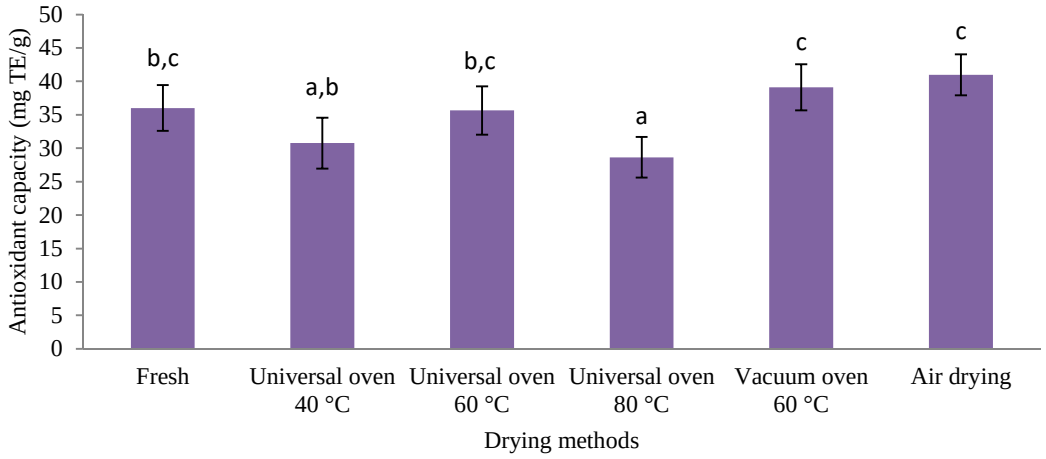

Figure 4. Effects of different drying methods on the antioxidant activity of *C. nutans* leaves. Data are reported as means ± standard deviations of triplicate experiments. Means with different letters (a-c) were significantly different ($p < 0.05$) according to Duncan's multiple range tests.

Effect on Antioxidant Activity

The results obtained in Figure 4 indicated that different drying methods have a significant influence on the antioxidant yield of *C. nutans* leaves ($p < 0.05$) from SFE extraction. For this study, the highest DPPH value at 41.00 ± 3.07 mg TE/ g was obtained from air-dried extract, followed by vacuum oven dried extract at 39.12 ± 3.45 mg Te/g. The total antioxidant activity is based on the monitoring of the DPPH free radicals absorbance value which will decrease in the presence of antioxidants. When antioxidant molecules react with DPPH free radicals, DPPH accepts a hydrogen atom from the antioxidant which results in the reduced form of DPHH [31]. Similar to the outcome by Hossain, et al. [21], highest antioxidant activity was obtained by air-dried samples, leading the study to conclude that air drying in ambient temperature causes the metabolically active plants to lose moisture slowly, which then triggered the plants to release phenolic compounds as part of a stress response. The lowest antioxidant activity was obtained from sample UO80 with 28.65 ± 3.02 mg Te/g. This result indicated that the drying method using high temperature resulted in thermal degradation of bioactive compounds in *C. nutans*. This similar outcome was also reported in a study by Pham, *et al.* [14], where a decrease in antioxidant capacity is observed when the oven temperature is increased from 80°C to 90°C.

CONCLUSION

In the present study, the overall results showed that different drying treatments resulted in significant variations ($p < 0.05$) on colour parameter, rehydration capacity and antioxidant activity of *C. nutans* leaves. The best colour characteristic which was the most similar in appearance to that of fresh samples, were obtained by vacuum oven drying at 60°C. In terms of rehydration capacity, drying at 80°C obtained the highest value. The highest DPPH value was obtained from air-dried extract at 41.00 ± 3.07 mg TE/ g. This study also showed that the colour parameter and rehydration capacity of samples did not represent the bioactive compound content of *C. nutans*.

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