The Effect of Fermentation and Drying on Clicanthus Nutans Lindai Extract Chemical Constituents and Bioactivity

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Abstract. In this study, two processes (fermentation and drying) were conducted on Clicanthus Nutans Lindau, which is well-known for its antioxidant activities. The aim of this research is to investigate the effects of these processes towards the Total Phenolic Content (TPC), antioxidant activity and the crude yield of the leaves. For the fermentation process, the fermentation time is varied for 6, 12, 24, 48 and 72 hours. For drying, oven drying (40, 45 and 50°C), freeze-drying and shade drying are investigated. Blanching was also conducted for oven dried samples (90°C for 1 minute). It was found that 12 hours fermentation produced the highest yield corresponding to Total Phenolic Content at 0.33% while 6 hours fermentation produced the highest Antioxidant Activity and yield at 2.218 x 10-10 mg/mL and 0.64%. On the other hand, 48 hours fermentation produced the highest overall extract yield at 0.64%. Analysis for antioxidant activity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteau method for Total Phenolic Content. Thermal inactivation kinetics (blanching) has been investigated for oven drying samples. Results showed that 40°C oven drying and 50°C oven drying with blanching has better retention of bioactive constituents. Blanching has no significant effect on phenolic content but has increased the crude extract for higher temperature oven drying. It has no positive effect on antioxidant activity. Drying is also a better processing method compared to fermentation as drying has slightly favorable results in all three aspects. Through comparison with extraction time of 60 minutes for 40°C oven drying and 6 hour fermented samples, yield of total phenolic content and overall yield of oven dried sample is higher than fermented sample by 4.14% and 0.39% respectively as well as showing better antioxidant activity.

1. Introduction
Medicinal herbs are fast gaining popularity in many countries [1]. Such products have become more widely available commercially, particularly in developed nations. Although modern medicine may exist side by side with such traditional practice, herbal medicine has often kept their popularity for historical and ethnic causes. According to the World Health Organization (WHO) figures, 80% of the world’s population depend on herbal medicine for primary health care [2]. Tea is considered as one of the most popular beverage consumed around the globe [3]. A growing body of research suggests that drinking tea actually helps to prevent cardiovascular disease, burn calories and ward off some types of cancer [4].

Today, tea is grown on a commercial scale in some three dozen countries and, in each country the product makes a substantial contribution to the economy of the agriculture sector. The thousands of
different varieties of teas available across the globe world only vary by the region it is grown, the time of year it is picked, and how it is manufactured. There are many types of teas available in the market, however the well known ones are commonly green tea, black tea, white tea, oolong tea, and pu-erh tea. They are all derived from the Camellia sinensis plant, a shrub native to China and India and they are produced based on the degree of fermentation [5].

All teas contain unique antioxidants called flavonoids which are antioxidants that help neutralize free radicals that can destroy cells and contribute to chronic diseases [6]. Caffeine and theanine are also found in teas whereby these two compounds affect the brain and heighten mental alertness [6]. Polyphenols are the secondary metabolites of plants and have a great potential as an alternative source of treatment of chronic diseases. The polyphenolic content of tea is mainly attributed to flavonoids including flavan-3-ols and flavanols, which account for 30% of the dry weight of fresh tea leaves. The major metabolites in tea leaves are flavonoids such as epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [7]. EGCG is an effective flavan-3-ol component that is known for its antioxidant and anticancer activities among all the flavonoids [7]. The simple substrates for example catechins are acted upon by the oxidative enzyme, polyphenol oxidase and peroxidase, to form theaflavins and thearubigins during fermentation [7].

Tea is a promising natural source for these bioactive ingredients which play a significant role in different nutritional and therapeutic effects of tea to delay the onset of risk factors associated with the development of diseases like cancer and diabetes [8]. It has been proposed to have antimutagenic, antiviral, anti-oxidant and anti-inflammatory properties in various biological systems [9].

Sabah Snake Grass is a herb that belongs to the acanthaceae family. Its scientific name is Clinacanthus nutans Belalai Gajah or Tarum Siam in Malay or Dandang Gendis in Indonesia. It can be commonly found in countries with tropical weather such as in Malaysia and Thailand. The leaves of Clinacanthus nutans Lindau have long been traditionally used in Thailand as an anti-inflammatory drug for the treatment of insect bites, herpes infection and allergic responses [10]. Recently, it was discovered that it possess anti-oxidant and anti-proliferative properties that are clinically proven effective against cultured cancer cell lines [8]. A research study carried out by Yong et al [8] has shown that it possess antioxidant and anti-proliferative properties against cultured cancer cell lines, suggesting an alternative adjunctive regimen for cancer prevention or treatment.

2. Experimental Setup

Two sets of procedures were carried out separately, namely fermentation and drying. The processed C. Nutans leaves will be extracted using the same parameters and analyzed.

2.1. Raw Materials Preparation

Sabah Snake Grass is obtained from the plot planted in Universiti Malaysia Sabah. The leaves are plucked and ensured to be free from damage. The leaves are rinsed with distilled water and pat-dried.

2.2. Fermentation procedure

3 grams of C. Nutans leaves are withered first and fermented after that. After fermentation, the leaves will be dried to halt the fermentation process. The leaves will then be extracted and analyzed accordingly.

2.2.1. Withering. The fresh leaves were withered in the incubator at 28°C in an incubator to bring about adequate physical and chemical withering. The leaves were withered till total moisture content is between 60%-70% of the initial moisture content of the leaves.

2.2.2. Fermentation. The sample was then crushed and wrapped in aluminium foil and then placed in ceramic beaker at the desired duration. The fermentation was conducted for different duration of fermentation (3, 6, 12, 24, 48, 72 hours) in an incubator at 30°C.
2.2.3. Drying. The leaves were then oven-dried at 40°C until the moisture content of tea leaves is between 3-5% to stop the fermentation activity [8]. This is the optimum range of temperature drying for C. nutans.

2.2.4. Extraction. There was only one extraction method used in this study; hot water extraction using distilled water. The fermented Sabah Snake Grass leaves were soaked in distilled water for 1 hour with magnetic stirrer for which the solvent to solid ratio (w/w) was 100:1. 300 g of distilled water at 80°C were used in the extraction. The experiment tested the effect of extraction time on Sabah Snake Grass for DPPH, Total Phenolic Content and Crude. Each experiments set were replicated for accuracy of results. Sampling was conducted at 5, 10, 15, 25, 40, 60 and 90 minutes.

2.3. Drying Procedure
A separate batch of C.Nutans leaves were conducted for drying. In this research, freeze drying, shade drying and oven drying was carried out. Blanching is also observed for the drying process.

2.3.1. Total Moisture Content of C. Nutans leaves. 5 grams of C. Nutans leaves are dried at 105°C for 24 hours according to the A.O.A.C. method. The weight before and after drying were measured and the total dry mass of the leaves are determined using the formula below. The total moisture content is the total mass subtracted with the total dry mass.

\[
\text{Moisture Content (\%) = \left[ \frac{M_i - M_f}{M_i} \right] \times 100\%}
\]  

Where \( M_i \) is the initial mass and \( M_f \) is the final mass.

2.3.2. Drying Kinetics. The drying kinetics for oven dried samples and shade dried samples are developed by drying the samples and weighing the samples at constant time intervals.

2.3.3. Blanching. Blanching is carried out for oven dried samples for 1 minute at 90°C. The samples are immediately immersed in ambient temperature distilled water to stop the blanching process. The samples are then oven dried accordingly.

2.3.4. Drying. The samples are oven dried at 40, 45 and 50°C, freeze dried and shade dried. The drying is carried out in duplicates. 3 grams of C. Nutans leaves were used for each sample.

2.3.4.1. Oven Drying. The samples are dried for 4 hours for 50°C, 7 hours for 45°C and 17 hours for 40°C, based on the drying kinetics.

2.3.4.2. Freeze Drying. The fresh leaves are frozen in a -20°C freezer overnight. The leaves are then placed into the freeze dryer for 48 hours at -50°C and a pressure of 500 mbar.

2.3.4.3. Shade Drying. The samples are placed in paper boxes with punctured holes to maximize air flow. The boxes are placed in well ventilated and airy areas but free from sunlight and rain.

2.4. Extraction.
The dried samples are placed in a teabag and extracted with 250 ml of distilled water at 80°C and extracted for 90 minutes. 3ml is sampled at the time intervals of 5, 10, 15, 25, 40, 60 and 90 minutes for analysis, similar to the extraction method of the fermentation process.

2.5. Analysis
The extracted samples were analysed using the methods shown below and the results are tabulated and compared between fermentation and drying.
2.5.1. **Crude Concentration.** 0.3ml of extract is taken from the 3ml sample that is extracted at every time interval. It is diluted into 3ml using distilled water. The samples for each time interval is analysed using the UV-Vis Spectrophotometer.

2.5.2. **Total Phenolic Content.** 0.2ml of extract is used for the total phenolic content analysis. Folin-Ciocalteau reagent is diluted 10 times. A sodium carbonate solution of 60g/L is also prepared. With 0.2ml of extract, 1.5ml of diluted Folin-Ciocalteau reagent is added and left for 10 minutes. After that, 1.5ml of the prepared sodium carbonate solution is added and left for 120 minutes. The samples are analysed using the UV-Vis Spectrophotometer.

2.5.3. **Total Antioxidant Activity.** 0.02% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution is prepared using 97-99% ethanol. 1ml of DPPH is added to 2.5ml of extract and left in the dark for 1 hour. The solution is analysed using the UV-Vis Spectrophotometer and the Radical Scavenging Activity is calculated based on the formula shown below.

\[
DPPH\text{ Radical Scavenging Activity (\%)} = \left(\frac{A_o - A_s}{A_o}\right) \times 100
\]  

(2)

2.5.4. **Overall Yield.** A selected sample extract was freeze dried and a calibration curve was made from freeze dried extract on UV-Vis Spectrophotometer to measure concentration. In each experiment, the same calibration curve was used to measure extract concentration. Overall yield is calculated based on the formula shown below:

\[
Yield = \frac{\text{volume of extract} \times \text{extract concentration}}{\text{weight of sample}}
\]  

(3)

3. **Results and Discussion**

3.1. **Fermentation Process**

The results of the analysis carried out from the fermentation process were discussed in this section.

3.1.1. **Crude Extract.** Figure 1 shows the average yield produced from Sabah Snake Grass. The yield for Sabah Snake Grass is calculated by multiplying the volume of solution left with the concentration of crude, both for every time interval to obtain the crude extract weight within the solution. The calculated extract weight is then divided by the amount of tea leaves used, which is 3g to obtain the yield (wt/wt %) produced. The calculations were done in a Microsoft Excel spread sheet.

Based on Figure 1, 40 minutes has been set as the cut-off point since the yield nearly reaches the equilibrium where most of the value compound could have been extracted. It can be observed that 48 hours fermentation produced the highest yield at a value of 0.62% while 72 hours fermentation produced the lowest yield. This may due to further enzymatic activities in sample which degrade desired compounds such as phenols and theaflavins in leaves after the optimum fermentation period.

The low yield for 12 hours fermentation during 10 minutes extraction may be due to solid diffusion factor that hinder the compound from diffusing out from the leaves.
3.1.2. Effect of Fermentation Duration on Sabah Snake Grass for Total Phenolic Content. For higher accuracy, the average values of yield corresponding to total phenolic content of dried Sabah Snake Grass extract at various duration of fermentation and extraction for both replicates was calculated and plotted as shown in Figure 1. 60 minutes has been set as the cut-off point as the yield started decreasing after that point assuming that it has nearly reach equilibrium where most of the value compound could have been extracted. 60th minute has been set as the cut-off point since the yield started decreasing. It can be observed that the highest yield was found to be 12 hours of fermentation peaking at 60 minutes with a value of 0.33%.

It can be said that most of the phenolic content have already been extracted for the first 60 minutes and started decreasing slightly onwards. In other words, high amount of phenolic compound is being extracted by hot distilled water during this period and started degrading after 60 minutes.

A previous study by Ariffin et al. [11] explained that tea fermentation induces polyphenols oxidation reactions by the enzyme of polyphenols oxidase which resulted in the breakdown of phenolic compounds as main contributor to the antioxidant activities. This may explain the inconsistencies of the results for the first replicate and second replicate. The duration of fermentation affects the level of phenolic compounds [12]. The result obtained indicated that time for tea fermentation is indeed quite crucial which may affect the level of phenolic compounds and potential antioxidant activities of the tea plants [13].

It can be concurred that 12 hours fermentation is the optimum time for fermentation of C. nutans since it is able to produce the high total phenolic content as well as yield.
3.1.3. Effect of Fermentation Duration on Sabah Snake Grass for Antioxidant Content (DPPH). For higher accuracy, the average of the IC50 values of the two replicates were calculated. The IC50 values (concentration of the extract that is able to scavenge half of the DPPH radical) are presented in Table 1. Based on Table 1, it can be observed that 6 hours fermentation recorded the lowest IC50 value which is an indicative of good antioxidant potential. While 48 hours fermentation recorded the highest IC50 value. Low IC50 value is indicative of good inhibition of the enzyme. The antioxidant potential of a compound can be attributed to its radical scavenging ability, and in order to evaluate the ability of the plant extracts to serve as antioxidants [14].

Based on Table 1, it can be observed that 6 hours fermentation produced the highest antioxidant activity since it has the lowest IC50 value compared to the rest. The IC50 values also increased with increasing fermentation time. This is in agreement with that study by Heong et al.[12], also observed that the longer fermentation in full-fermented of tea had lower level of total antioxidant activities than in partial-fermented tea.

The extracts for various fermented time showed a propensity to extinguish the free radicals, as indicated by the dose dependent increase in percentage inhibition. This corresponded to a rapid decrease in absorbance in the presence of a plant extract, indicating high antioxidant potency of the extracts in terms of electron or hydrogen atom-donating capacity [15].

Most of the antioxidant potential of medicinal plants is due to the redox properties of phenolic compounds, which enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers [16]. In addition, the hydrogen-donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids enable them to undergo a redox reaction, which in turn, help them scavenge free radicals [17].

Table 1. Antioxidant activity represented by IC50. Various Duration of Fermented Sabah Snake Grass Extract, measured by DPPH Radical Scavenging Test by taking the average.

| Duration of Fermentation | DPPH Test IC50 (mg/mL) |
|--------------------------|-----------------------|
| 6 Hours                  | 2.218 x 10^{-10}      |
| 12 Hours                 | 4.942 x 10^{-0}       |
| 24 Hours                 | 12.273 x 10^{-0}      |
| 48 Hours                 | 2.403 x 10^{11}       |
| 72 Hours                 | 22.414 x 10^{0}       |

Figure 3 illustrates the overall yield produced corresponding to the total antioxidants produced against the time of extraction. 60 minutes has been set as the cut-off point since the yield for most fermentation time started decreasing thereafter, indicating that all constituents containing in the fermented Sabah Snake Grass tea has been extracted. It can be observed that both 6 hours fermentation and 48 hours fermentation produced the highest yield with a value of 0.64% and 0.64% respectively, with 48 hours fermentation registered a slightly higher value. On the other hand, 72 hours fermentation registered the overall lowest yield.
3.1.4. Analysis of TPC per Crude in Sabah Snake Grass. Analysis of TPC per Crude is to see the relation between TPC and crude in fermentation of C. nutans. Based on the plot, it can be determined at which point does the crude and total phenolic content is released. Figure 4 shows the overall TPC per Crude activity of various fermented Sabah Snake Grass. The range of yield (wt/wt %) of TPC/Crude activity can be observed to be between 0.39% to 1.91%.

3.2. Drying Procedure

3.2.1. Total Moisture Content of C. Nutans leaves. The total moisture content is found to be 79.61±0.0424% based on duplicate samples.

3.2.2. Drying Kinetics. The drying kinetics are shown in Figure 5. It is shown that for 40°C sample, the drying time is 17 hours, 7 hours for 45°C and 4 hours for 50°C. The shade dried sample takes 3 days to dry completely, which is shown in Figure 6.
3.2.3. Crude Extract. Based on Figure 7, it can be observed that the graphs all follow the first order curves, increasing rapidly at the first 20 minutes of extraction, then starts to slow down until 40 minutes. After 60 minutes, the curve starts to flatten out, which means that it has started to stabilize, and also that all of the extracts have been extracted. Hence, the 60 minute point is the equilibrium point for the crude concentration. From the graphs above, it can be seen that the total yield ranges from around 0.8% to 1.1% (at the 60 minute point).

As shown in Figure 7, the trend is observed to be as follows:
40°C oven > 50°C oven blanched > Freeze dried > 50°C oven > 45°C oven > 45°C oven blanched > 40°C oven blanched > 50°C oven

Comparing the trends shown above, there may be a slight difference in the arrangements, but referring to the plots of the yields shown, it can be generalized that for non-blanched oven samples, the lower the temperature the higher the yield. However for blanched oven samples, the higher the temperature, the higher the yield, which is more desired. Whereas the freeze dried samples have a higher yield compared to the shade dried samples. Both the shade dried and freeze dried samples have relatively high yields as compared to the oven dried samples. Based on the trend above, it can be noticed that blanching had an effect on the extractability of crude. Blanching is used for enzyme inactivation and retention of the quality of fresh plants. Although there may be possibilities that the volatiles and some constituents may leech into the water from the blanching process, the structure of the leaves for each plant and their constituents vary from one another [18]. Hence, blanching may have a different effect on different samples. For the case of C.Nutans, the change was visible in crude
samples. Hence this may mean that blanching was unfavorable for low temperature drying but increasingly beneficial for constituents retention for increasing temperature.

3.2.4. Total Phenolic Content. Based on Figure 8, it has a same first order curve, with rapid increase at the first 30 minutes, slowing down at 40 minutes and eventually stabilizes after 60 minutes. This has the same pattern as the curves for yield of crude extracted. It can be seen that the yield of phenolic content ranges from 0.25% to 0.35%. The 45°C oven blanched curve has a decrease in phenolic yield, different from the other drying methods. This may be due to the deterioration of phenolic content after extraction of 80°C for an extended 30 minutes. At 60 minutes, the descending order of phenolic content is as shown below.

Shade dried > 50°C oven blanched > 40°C oven > Freeze dried > 40°C oven blanched > 50°C oven > 45°C oven > 45°C oven blanched

Although there may be a difference in the arrangement in the trends for both replicates, generally for the shade dried, freeze dried, 40°C oven-dried and 50°C oven-dried are quite close in yield. It can also be seen that there is a similar trend in descending order for both blanched and not blanched oven samples.

Oven-dried samples
40°C oven > 50°C oven > 45°C oven
Blanched oven-dried samples
50°C oven blanched > 40°C oven blanched > 45°C oven blanched

Based on Figure 9, the 45°C oven drying has the lowest phenolic yield for both. Hence this should be looked into. For both cases, the 45°C drying has the lowest yields. Based on a research, convective drying resulted in a lower content of rosmarinic acid due to the long time exposure to oxygen during the drying process [19]. They have also proposed in another research that rosmarinic acid degradation is associated to the temperature of the air for drying [20]. It can be observed from the bar charts above, for both blanched and unblanched, the phenolic contents drop from 40°C to 45°C then increases at 50°C which can be explained by the findings stated above. Although 40°C drying has the longest drying time and it is most exposed to oxidation, less volatile constituents were evaporated due to its lower temperature. Hence it could be concluded that the phenolics in the extract are heat-sensitive. However, 45°C drying has higher temperature and longer drying time than 40°C. Hence, 45°C drying has shown to have the lowest phenolic yield among all drying methods. 50°C however has an increased phenolic content. This indicates that more phenolic contents are released by higher heat. It has also shown that blanching has no significant effect on the phenolic contents as there are no differences in the trend between the blanched and unblanched samples. The freeze dried and shade dried samples have a relatively high phenolic content compared to the oven dried samples. Freeze-dried is a favorable drying method for samples possessing thermostable.

From the trends above, the shade dried samples have very high phenolic contents. This corresponds to the research done on different herbs where the air-dried samples have the highest total phenolic content [21]. As the leaves were dried using the longest period of time amongst all drying methods, metabolically active plants lose moisture slowly and sensed moisture loss as a stress. As a defence mechanism, plants in general produce phenolic compounds as a stress response [22].
3.2.5. Total Antioxidant Activity. From Figure 11, the radical scavenging activity ranges from 73%-97%. At 10 minutes, the radical scavenging activity ranges from 85%-97%. It reaches an equilibrium point at around 60 minutes. It can be observed that the freeze drying samples have lower than expected antioxidant contents, as many researches stated that freeze drying has been one of the best ways of preservation [23-25]. It has also been said to show a less pronounced damaging effect on tissue structure than other drying methods [26]. From the graphs of radical scavenging activity with respect to extraction time below it can be observed that the freeze-dried method does not yield the highest radical scavenging activity which corresponds to the antioxidant contents in C. Nutans leaves. This finding corresponds to what have been found on the research on lemon balm, where the essential oil contents of dried samples were far lower than dried samples particularly samples of freeze drying and convective drying. They suggested that by processing the leaves at a pressure lower than atmospheric pressure is one of the causes of essential oil deterioration because of the volatility, polarity and chemical structure of the constituents in the leaves [27]. The decreasing of radical scavenging activity
may due to the degradation of compounds that scavenge free radicals in hot water extraction condition as the extraction period increases.

![Radical Scavenging Activity with respect to Time](image1)

**Figure 11.** The Radical Scavenging Activity with respect to Time.

3.2.6. **Total Phenolic Content per Crude.** Based on Figure 8, it can be seen that generally the phenolic contents are extracted faster than the crude. For both replicates, the 40°C oven dried samples and to 50°C oven dried samples, the phenolics are extracted faster compared to other samples. For all the samples, after 40 minutes, the graphs start to flatten out meaning that the phenolics have mostly been extracted by that time. So the equilibrium ratio of total phenolic content to crude is in the range of 0.4 to 0.7 for all samples. The phenolics exit faster due to the structure of the leaves that contains these compounds may be more vulnerable to heat.

![Total Phenolic Content per Crude with respect to Time](image2)

**Figure 12.** The Total Phenolic Content per Crude with respect to Time.

3.3. **Comparison between Drying and Fermentation for Optimum Condition**

The drying of C. nutans leaves was carried out by the other researcher who is studying the effect of drying on C. nutans leaves. The drying and fermentation data were compared at optimum condition.

3.3.1. **Total Phenolic Content.** Figure 13 shows the total phenolic content for fermentation and drying of C. nutans leaves at optimum condition whereby 12 hours fermentation data was compared against drying datas of C. nutans at 40°C. It can be observed that the total phenolic content for fermented C. nutans is higher compared to drying only for the first 40 minutes. During fermentation, biotic and abiotic stress factors such as wounding, low temperature and pathogen attacks may trigger defences mechanisms by the synthesis of phenylpropanoid compounds such as flavonoids, isoflavonoids, psoralens, coumarins, phenolic acids, lignin and suberin [28-29].
By comparing at the cut-off point ($t=60$ mins) for various fermented times of C. nutans, the yield corresponding to total phenolic content for drying at 40°C is significantly higher compared to fermentation as shown in Table 2. The findings for this is supported by Abu Bakar et al. [30] where the unfermented leaves of Strobilanthes crispus showed better antioxidant and antiradical activities as compared to fermented leaves due to deterioration of phenolics content during fermentation.

Other previous studies also reported that some phenolics compound present in leaves might be converted or degraded during fermentation [31-34]. The formation of colour and flavour compounds during fermentation of C. nutans leaves also might reduce the polyphenol concentration [35].

![Comparison of Total Phenolic Content between Drying and Fermentation.](image)

**Figure 13.** Comparison of Total Phenolic Content between Drying and Fermentation.

| Yield (%) | Drying Duration of Fermentation (Hrs) |
|-----------|--------------------------------------|
|           | Temp (40°C)                           |
|           | 6 12 24 48 72                          |
| 4.80      | 0.64 0.55 0.52 0.64 0.50               |

3.3.2. Antioxidant Activity (DPPH). The drying and fermentation data are compared at optimum condition. Table 4 shows the comparison of antioxidant activity, represented by IC50 of various duration of Fermented Sabah Snake Grass extract, measured by DPPH Radical Scavenging Test between Drying at 40°C and 6 hours fermentation. Based on Table 4, the IC50 values for drying of C. nutans leaves is significantly lower compared to the fermentation of C. nutans leaves. A low IC50 value is indicative of good inhibition of the enzyme.

The antioxidant potential of a compound can be attributed to its radical scavenging ability, and in order to evaluate the ability of the plant extracts to serve as antioxidants [14]. The findings for this experiment suggested that there is a reduction in antioxidant capacity in tea fermentation as supported by Kim et al. [5]. The decline of other phytochemicals including flavonol, glycosides, caffeine, saponin and ascorbic acid by oxidation or heat exposure during the fermentation process might lead to the reduction of antioxidant capacity [15]. This implies that drying method on C. nutans can preserve more antioxidant on this C. nutans leaves.
Table 3. Comparison of Antioxidant activity, represented by IC50 of Various Duration of Fermented Sabah Snake Grass Extract, measured by DPPH Radical Scavenging Test between Drying and Fermentation.

| Method       | DPPH Test IC50 (mg/mL) |
|--------------|------------------------|
| Drying       | 1.670 x 10^-11         |
| Fermentation | 2.218 x 10^-10         |

Table 4. Comparison of Antioxidant activity, represented by IC50 of Various Duration of Fermented Sabah Snake Grass Extract, measured by DPPH Radical Scavenging Test between Drying and Fermentation at optimum condition. (t=60 mins)

| IC50 (mg/mL) | Drying Duration of Fermentation (Hrs) |
|--------------|--------------------------------------|
| Temp (40°C)  | 6  12  24  48  72                   |
| 1.670 x 10^-11 | 2.218 x 10^-10   | 4.942 x 10^0   | 12.273 x 10^0 | 2.403 x 10^11 | 22.419 x 10^0 |

3.3.3. Crude Yield. Figure 14 shows the comparison of extract yield between fermentation and drying of C. nutans at optimum condition whereby the average yield of 48 hours fermentation data was compared against drying conducted at 40°C. It can be observed that the yield produced by drying is higher than fermentation from 20 minutes onwards. It is also significantly higher when compared with the yield produced from fermentation at various times as shown in Table 5. During drying, enzymatic activities in samples were halted which prevents the deterioration of phenolic compounds which results in higher phenolic yield compared to those undergoes fermentation [21]. The yield range of these experiments is within the range of most herbal extracts which are between 1.0 to 5% yield.

Fermentation of tea leaves involves the chemical breakdown of substance by bacteria, yeast or other microorganism. There is a possibility that some compound present in C. nutans tea leaves might have been converted or degraded during fermentation, hence resulting in a lower yield produced compared to drying. Further study is required to be carried out in order to examine the reasons behind this phenomenon.

Figure 14. Comparison of Extract Yield between Drying and Fermentation.
4. Conclusion

In this study, two processes which are drying and fermentation are conducted on C. Nutans leaves to investigate how these processes affect the total phenolic content, antioxidant activity and the yield of crude. The fermentation time is varied for the fermentation process while different drying methods were carried out for the drying process. Blanching was also conducted on the drying process. For the fermentation process, to produce a tea with high phenolic content as well as highest yield, C. nutans leaves will need to be fermented for 12 hours. On the other hand, to produce a tea with high antioxidant content as well as the highest yield, C. nutans leaves will need to be fermented for 6 hours. To produce with the overall highest yield without taking the antioxidant and total phenolic content into consideration, 48 hours fermentation will be sufficient. It can also be concurred that C. nutans tea leaves should be brewed for 40 to 60 minutes in order to reap the maximum antioxidant and phenolic compounds. For the drying process, the leaves were found to dry completely when oven dried for 4 hours for 50°C, 7 hours for 45°C and 17 hours for 40°C. The extraction with time for crude and phenolic contents similar to a first-order kinetics graph and reaches equilibrium at around 60 minutes. The yield of crude was found to be in the range 0.8%-1.1% for all drying methods and has a concentration of 0.0942mg/ml – 0.1302mg/ml. 40°C oven drying and 50°C oven drying with blanching is more favorable for crude. All the drying methods yields around 0.25%-0.35% of phenolic content equivalent to 0.0016mg/ml GAE – 0.0020mg/ml GAE. The best methods for favorable phenolic retention are shade drying, 40°C oven drying and 50°C oven drying with blanching. The IC50 values for antioxidant activity shows that with higher processing temperature, the better the antioxidant content, but more research has to be carried out on this for a better justification as the results were not consistent. Blanching has an effect of increasing crude extract with increasing temperature, whilst it is vice versa without blanching. There is no significant effect of blanching towards the phenolic content of C. Nutans. When compared to fermentation based on the results from another researcher, drying showed a more favorable edge in all three aspects. In a nutshell, 40°C oven drying and 50°C oven drying with blanching showed more favorable results for both crude and phenolic content. There were no significant differences in the radical scavenging activities of both. Hence, 40°C oven drying and 50°C oven drying with blanching are the best methods of processing C. Nutans with high bioactive constituent retention. Further research should be carried out at different blanching temperatures and higher oven drying temperatures.

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