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Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah Snake Grass (*Clinacanthus nutans* Lind.) leaves

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Abstract

Clinacanthus nutans (Burm. F.) Lindau or locally known in Sabah, Malaysia as 'Sabah Snake Grass' has been ethnobotanically used to treat various diseases in Asian countries. This study was conducted to determine the total phenolics content (TPC), flavonoids content (TFC) and antioxidant activity of herbal teas developed from *C. nutans* leaves with different drying techniques (microwave-oven dried and freeze dried) and infusion time (1, 2, 5, 10, 15 and 20 min). Ferric reducing/antioxidant power (FRAP) assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2, 2-diphenyl-1-pycryl-hydrazyl (DPPH) free radical scavenging assays were used to investigate the antioxidant capacity. The highest TPC of herbal tea was observed in 20 min infusion of unfermented microwave-oven dried leaves (177.80 ± 19.10 mg TAE/L), while the highest TFC was observed in 10 min infusion of fermented microwave-oven dried leaves (22.13 ± 1.53 mg CE/L). Short infusion times from 5 min to 15 min were able to extract high amount of phenolics compounds. Unfermented tea contained higher TPC content ($P < 0.05$) as compared to fermented tea, while, TFC showed no significant difference between both types. Freeze dried infusion shows no significant difference ($P > 0.05$) as compared to microwave-oven dried for TPC, TFC and antioxidant capacity. Moderate and low correlation was observed between TPC and FRAP values (r = 0.507) and between TFC and ABTS values (r = 0.256). Preparation of *C. nutans* herbal tea as potential natural antioxidant source can be used as a basic reference for future research on the dietary intake of these herbal teas.

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Introduction

For the past 4000 years, tea developed from the leaves of true tea plant, *Camellia sinensis*, has been rated as second mostly consumed beverage after water (Weisburger, 1997; Ergüder *et al*., 2008). Basically, tea can be found in many different forms depending on the method of preparation and level of fermentation such as green (unfermented), oolong (semi-fermented) and black (fermented) tea. Numerous scientific and detailed researches on tea have revealed the health promoting properties including prevention of chronic diseases such as cancer and cardiovascular disorder (Oak *et al*., 2005; Yang and Wang, 2010). Occurrence of chronic diseases were recognized to be associated with the oxidative stress, where the reactive oxygen species (ROS) and reactive nitrogen species (RNS) including free radicals were continuously produced in human cells and led to oxidative damage to cell components (Bagchi and Puri, 1998; Valavanidis *et al*., 2013). In concern by that, there is an increasing interest in antioxidants; chemical compounds that possess the ability to neutralize these free radicals in the body by reducing or scavenging its activities (Pisoschi and Negulescu, 2011). Antioxidants are known to play a key role in the protective influence exerted by dietary plants. There are numerous antioxidants reported in dietary plants such as phenolics compounds, flavonoids, carotenoids, benzoic acids and derivatives, coumarins, proanthocyanidins stibenes, lignans and lignins (Lindsay and Astley, 2002). Among many dietary plants, *C. sinensis* tea contains high amounts of catechin and other polyphenol that exhibit powerful antioxidant activities (Dufresne and Farnworth, 2001). However, in recent years,

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many researchers have focused their investigation on developing herbal teas based on potential plants other than *C. sinensis* (Abu Bakar *et al.*, 2005).

Clinacanthus nutans (Burm. f.) Lindau (family Acanthaceae) or locally known as 'Sabah snake grass' or 'Belalai Gajah' in Malaysia is native to tropical Asia and generally known for its medicinal properties. In Malaysia, this plant has been used traditionally for its potential to cure cancer (Roosita *et al*., 2008). In Thailand, fresh leaves are believed to cure insect and snake bite, skin rashes, herpes simplex virus (HSV) and varicella-zoster virus (VZV) lesions (Sakdarat *et al*., 2009). Meanwhile, people in Indonesia use it to treat diabetes and dysentery (Hariana, 2008). The ethanolic extract of *C. nutans* leaves was reported to exhibit antioxidant activity and protective effect against free radical-induced hemolysis (Pannangpetch *et al*., 2007). Numerous previous findings have reported the influence of factors such as sample preparation and processing, as well as the extraction procedures on the phenolics yield (Chan *et al.*, 2009; Lin *et al*., 2010; Chong and Lim, 2011; Ji *et al*., 2012). Drying techniques are important in sample preparation and preservation. By reducing the moisture content less than 15%, drying helps to prevent any microbial growth. In fact, efficient drying techniques will enhance the quality of dried product such as aroma and appearance by hindering any biochemical changes (Rabeta and Lai, 2013). Drying can be thermally such as sun dry and oven dry or non-thermal using freeze drying technique. Generally, hot-air drying usually used as conventional method in the food industries, however, it can brings adverse effects to its plant product quality due to high temperature and long drying time (Sharma and Prasad, 2003). Meanwhile, microwave-vacuum drying and freeze drying are considered as better methods to minimize the nutrient loss (Rabeta and Lai, 2013). Although, there are many studies have been carried out on the effect of these drying techniques on plant product quality, there a little information available on the effect of using microwave-oven drying in household use. Therefore, this present study was conducted to investigate the effect of different drying techniques (thermal: microwave-oven drying and non-thermal: freeze drying) and infusion times to its antioxidant activity, total phenolics and flavonoids content of unfermented and fermented herbal tea of *C. nutans*.

Materials and Methods

Chemicals and reagents

All chemicals used in this study were analytical grade and purchased from Sigma (USA), Merck (Germany), Fluka (USA) and ThermoFisher Scientific (USA). For leaves drying, microwave oven (Samsung Microwave Oven MW71E) and freeze drier (Labconco Freezone 12 Liter Freeze Dry System) were used. All absorbance measurements were made using a MULTISKAN GO-1510 (ThermoScientific) spectrophotometer.

Plant materials

Clinacanthus nutans plant was collected from Ranau, Sabah Borneo which is located in East Malaysia. Plant was identified and authenticated by Mr. Johnny Gisil (botanist) from Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. Voucher specimen was scientifically documented (LusiaBorh01) and deposited in the plant herbarium of BORNEENSIS (BORH). One kilogram of fresh intermediate leaves of C. nutans ($2^{nd} - 8^{th}$ axis) were collected and rinsed with distilled water to remove dust, soil and insects. Leaves were then blotted with tissue papers to absorb water and allowed to dry for 10 min in room temperature (25 ± 1°C).

Preparation of unfermented herbal tea

Preparation of unfermented herbal tea of *C. nutans* was based on the preparation of *C. sinensis* green tea according to Chan *et al.*, (2007) and Wang *et al*., (2000) with modification. The collected leaves (0.5 kg) were steam blanched as a pretreatment to inactivate the degradative enzymes for 30 sec at 98 ± 5°C and then immediately immersed in ice water bath for 30 sec to prevent the leaves from overcooked. The leaves were grounded using a blender for 5 sec into relatively small particle size and divided into two parts for further drying using microwave-oven dry and freeze dry technique, respectively. The leaves were dried at 600 W for 5 min using a microwave-oven until the moisture content was less than 4%. Meanwhile, for the freeze drying technique, the fermented leaves were first frozen under -80°C for 48 hours before subjected to freeze drier for 48 hours.

Preparation of fermented herbal tea

Preparation of the fermented leaves of *C. nutans* was adapted from the preparation of *C. sinensis* black tea according to Subramanian *et al.* (1999) with modification. The collected leaves (0.5 kg) were spread in a single layer on a tray and left at room temperature (25 ± 1°C) for 18 hours for withering process to obtain about 70% of moisture content. Withered leaves were grounded into relatively small particle size using a blender for 5 sec. Blended leaves

were sprayed with distilled water in 1:1 (w/v) ratio before undergone oxidation-fermentation process for 5 hours at 25 ± 1°C. The fermented leaves were further dried in the same procedure as preparation of unfermented leaves.

Preparation of samples infusion

In order to mimics household brewing conditions, herbal tea was prepared using hot boiling water extraction procedure. Each 2.0 g of dried unfermented and fermented leaves of C. nutans were infused in 200 ml boiled distilled water (100°C) and continuously stirred for 2 min using a magnetic stirrer under 300 rpm. The infusion left to cool to specific infusion time (1, 2, 5, 10, 15 and 20 min) before filtered using a filter paper (Wathman NO.4). Two commercial teas ("BOH Green Tea" and "SABAH Black Tea") were used as comparison to *C. nutans* herbal tea. These teas were locally purchased and infusions were prepared with the same infusion times as *C. nutans* herbal tea.

Determination of total phenolic content

Total phenolic content (TPC) of infusions were determined using Folin Ciocalteu's colorimetric method as described by Velioglu, *et al*. (1998) with slight modification. About 1 ml of each infusion was mixed with 0.4 ml distilled water and 0.75 ml of 10-fold diluted of Folin-Ciocalteu's reagent. The mixture was left stand for 5 min at 22 °C before added with 0.75 ml sodium bicarbonate (60 g/L) solution and allowed to stand in a dark room for 90 min at 22°C. The absorbance of all infusions was measured at 725 nm using spectrophotometer. TPC were quantified based on standard curve with various concentrations of tannic acid solutions (0 − 100 µg/ml) and expressed as milligram tannic acid equivalent per liter (mg TAE/L) with standard curve equation of $y = 0.001x + 0.056$ ($R^2 = 0.993$).

Determination of total flavonoid content

Total flavonoid content (TFC) of infusion was determined using aluminium chloride method as described by Zhizhen *et al*. (1999). 1 ml of each infusion was mixed with 4 ml distilled water, 0.3 ml of 5% sodium nitrate, 0.6 ml 10% aluminium chloride solution and 2 ml of 1 M sodium hydroxide. The absorbance of mixture was measured at 510 nm using spectrophotometer. TFC was expressed as milligram (-/+) catechin equivalent per liter (mg CE/L) based on the standard curve of various concentration of (-/+) catechin (0 - 100 µg/ml) prepared with a calibration equation of $y = 0.0002x + 0.046$ ($R^2 = 0.999$).

Ferric reducing/antioxidant power (FRAP) assay

The free radical reducing power of tea infusions were assessed using the Ferric reducing/antioxidant power (FRAP) assay as described by Benzie and Strain (1996) with slight modification. A total of 3.0 ml FRAP reagent (300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s- triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio) was added to a cuvette and a blank reading was taken at 593 nm using spectrophotometer. A total of 100 µl of tea infusion and 300 µl of distilled water were added to the previous cuvette. A second reading at 593 nm was taken after 4 min of incubation. The changes in absorbance after 4 min from initial blank reading were compared with standard curve. The FRAP values were determined using the standard curve of Fe (II) concentration generated from various concentration of ferrous sulphate solution (0 − 100 ug/ml) with standard curve equation of Fe (II), $y = 0.0004x + 0.002$ ($R^2 = 0.997$), where y is the absorbance value and x is the concentration of Fe (II) in µg/ml. The final result was expressed as the concentration of antioxidant having a ferric reducing ability in 1 liter of infusion (mg/L).

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) cation radical decolorization assay

The scavenging activity of infusions was assessed by ABTS radical cation decolorization assay as previously described by Re *et al.*, (1999). The incubation of ABTS solution (7 mM) and potassium persulfate (2.45 mM) mixture for 15 hours in the dark generate an ABTS free radical cation solution. The mixture then diluted with distilled water in order to obtain absorbance of 0.7±0.02 A at 734 nm as working ABTS solution. Each 200 µL of infusion were added to 2 ml of working ABTS solution and vortex for 45 seconds before measured the absorbance at 734 nm using spectrophotometer. ABTS values was determined based on a standard curve with various concentrations of ascorbic acid (0 − 100 µg/ml) with standard curve equation of ascorbic acid was $y=0.006x + 0.213$ ($R^2 =0.990$). The final result was expressed as milligram ascorbic acid equivalent antioxidant capacity in 1 liter of infusion (mg AEAC/L).

2, 2-diphenyl-1-pycryl-hydrazyl (DPPH) free radical scavenging activity

Free radical-scavenging activity infusions were determined by measuring the disappearance of violet color of stable free radical DPPH free radical solution as described by Mensor *et al*. (2001). Briefly, 2.5 ml of each infusion of varying concentration (0.1, 0.2,

Table 1.Total phenolics content of *Clinacanthus nutans* herbal tea

Type	Infusion time	Drying technique	
		Freeze Dried	Microwave-oven dried
Unfermented *C. nutans* herbal tea[1]	1	132.60 ± 18.57^{a}	131.24 ± 8.71^{a}
	2	140.08 ± 12.23^{ac}	143.92 ±18.76^{ac}
	5	143.96 ± 14.44^{ad}	143.56 ± 8.95^{ad}
	10	162.48 ± 12.58^{bcde}	164.48 ± 14.37^{bcde}
	15	158.48 ± 15.11^{aef}	146.80 ± 11.77^{ae}
	20	160.68 ± 5.49^{bcdf}	177.80 ± 19.10^{b}
Fermented *C. nutans* herbal tea[2]	1	88.56 ± 4.40^{a}	101.48 ± 15.79^{a}
	2	96.12 ± 15.55^{ac}	111.2 ± 13.59^{ac}
	5	105.88 ± 14.74^{ad}	137.08 ± 18.41^{bcd}
	10	108.72 ± 8.94^{ae}	136.92 ± 7.43^{bce}
	15	110.40 ± 7.68^{bcde}	134.52 ± 26.38^{ade}
	20	114.42 ± 8.21^{bcde}	105.60±16.02^{ade}
BOH Green tea[3]	1	1958.72 ± 121.63^{a}	
	2	2019.84 ± 146.29^{a}	
	5	2029.88 ± 117.37^{a}	
	10	2038.44 ± 117.37^{a}	
	15	2096.20 ± 173.81^{a}	
	20	2050.20 ± 104.09^{a}	
Sabah black tea[4]	1	1210.88 ± 75.71^{a}	
	2	1320.08 ± 90.21^{a}	
	5	1379.32 ± 95.92^{a}	
	10	1269.68 ± 95.92^{a}	
	15	1319.68 ± 123.74^{a}	
	20	1315.96 ± 90.62^{a}	

Mean ± S.D. (n = 5), Same superscripted number or letter within vertical columns are not significantly different (P > 0.05), TPC values were expressed in milligram tannic acid equivalent per liter (mg TAE/L)

0.4, 0.6, 0.8 and 1.0 mg/ml) was mixed with 1 ml of DPPH methanol solution and allowed to stand for 30 min in dark before measured the absorbance at 517 nm. The ability of the infusions to scavenge the DPPH free radical was calculated as follow:

$$\text{Scavenging activity (\%)} = [1- ((A_{sample} - A_{empty}) / A_{control}))] \times 100\%$$

where, A_{sample} is the absorbance of test samples (DPPH solution with samples), A_{empty} is the absorbance of samples only (sample without DPPH solution) and $A_{control}$ is the absorbance of control (DPPH solution without sample).

Statistical analysis

All data were analyzed using SPSS statistical 21 and expressed as means ± standard deviation (S.D.) of five replicate analyses in five independent experiments. One-way analysis of variance (ANOVA) followed by Tukey HSD (high significant difference) multiple range test was carried out to determine the significance between means. $P \leq 0.05$ level was set as statistical significant level. Pearson's correlation test was used to test correlation between phytochemicals contents and antioxidant activities of infusions.

Results and Discussion

Total phenolic (TPC) and flavonoids content (TFC)

As shown in table 1, total phenolics contents (TPC) were expressed as mg tannic acid equivalent/L of tea infusion ranged from the lowest values 88.56 ± 4.40 mg TAE/L to highest 2096.20 ± 173.81 mg TAE/L. For unfermented *C. nutans* herbal tea, the highest TPC was in 20 min infusion of microwave-oven dried leaves (177.80 ± 19.10 mg TAE/L). The TPC values were increased at 5 min infusion time and significantly higher (P < 0.05) at 10 min of infusion in both drying techniques. For fermented *C. nutans* herbal tea, the highest TPC was in 5 min infusion of microwave-oven dried leaves (137.08 ± 18.41 mg TAE/L). The TPC values were increased and significantly higher at 15 min and 5 min compared to early infusion time for freeze-dried and microwave-oven dried, respectively. The TFC of tea infusions in Table 2 were expressed in mg catechin equivalent/L of tea infusion ranged from 14.57 ± 0.42 mg CE/L to 340.22 ± 21.43 mg CE/L. For unfermented *C. nutans* herbal tea, the highest TFC was recorded in 10 min infusion (19.85 ± 0.90 mg CE/L) in freeze dried leaves. The TFC values were significantly higher in 10 min infusion compared to early infusion time for both freeze-dried and microwave-oven dried leaves. For fermented *C. nutans* herbal tea, the highest TFC was recorded at 10 min infusion time (22.13 ± 1.53 mg CE/L) in microwave-oven dried leaves. The TFC values were significantly higher at 10 min and 5 min compared to early infusion time for freeze-dried and microwave-oven dried, respectively. Due to relatively small particles and small surface area of dried leaves, shorter infusion time as 5 - 15 min were able to extract the phenolics and flavonoids compounds and shows similar content even for 20 min of infusion time.

Unfermented *C. nutans* herbal teas were proved to be significantly higher (P < 0.05) of TPC as compared to fermented *C. nutans* herbal tea, but

Table 2.Total Flavonoids content of *Clinacanthus nutans* herbal tea

Type	Infusion time	Drying technique	
		Freeze Dried	**Microwave-oven dried**
Unfermented *C. nutans* herbal tea[1]	1	17.25 ± 0.39^{abd}	14.57 ± 0.42^{a}
	2	17.00 ± 1.27^{ace}	16.43 ± 0.47^{ab}
	5	17.55 ± 2.12^{abd}	14.87 ± 0.72^{a}
	10	19.85 ± 0.90^{bd}	17.40 ± 0.70^{b}
	15	17.10 ± 1.57^{acd}	17.40 ± 0.83^{ab}
	20	19.73 ± 1.40^{bd}	16.47 ± 3.75^{ab}
Fermented *C. nutans* herbal tea[1]	1	17.50 ± 2.43^{acd}	15.70 ± 1.42^{a}
	2	14.80 ± 2.50^{ad}	17.33 ± 0.61^{ac}
	5	17.88 ± 1.28^{acd}	21.45 ± 2.67^{b}
	10	17.85 ± 0.95^{bc}	22.13 ± 1.53^{b}
	15	17.70 ± 1.57^{ad}	19.85 ± 3.42^{bcd}
	20	18.08 ± 0.79^{bcd}	16.13 ± 0.36^{ad}
BOH Green tea[2]	1	309.24 ± 9.56^{a}	
	2	327.44 ± 8.28^{ac}	
	5	324.86 ± 9.70^{ac}	
	10	321.28 ± 11.90^{ac}	
	15	340.22 ± 21.43^{bc}	
	20	313.26 ± 24.06^{ac}	
Sabah black tea[3]	1	204.54 ± 7.73^{a}	
	2	228.30 ± 11.02^{ac}	
	5	223.32 ± 10.05^{ac}	
	10	209.50 ± 13.54^{ac}	
	15	223.72 ± 17.25^{ac}	
	20	230.98 ± 18.14^{bc}	

Mean ± S.D. (n = 5), Same superscripted number or letter within vertical columns are not significantly different (P > 0.05), TFC were expressed in milligram catechin equivalent per liter (mg CE/L)

lower than the commercial teas tested. However, the TFC of unfermented *C. nutans* herbal tea were not significantly different (P > 0.05) than fermented *C. nutans* herbal tea and lower than the commercial teas tested. The TPC and TFC of "BOH Green Tea" (unfermented) were significantly higher (P < 0.05) than the "SABAH Black Tea" (fermented). The inactivation of degradative enzyme (polyphenol oxidase) during steam blanching prevents the oxidation of polyphenols, thus retaining more phenolics in unfermented tea (Belitz *et al*., 2009). The formation of color and flavor compounds during fermentation of *C. nutans* leaves also might reduce the polyphenol concentration (Vanderhaegen *et al*., 2003). Present findings of this herbal tea was supported by Abu Bakar *et al*. (2006) 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 that present in leaves might be converted or degraded during fermentation (Joubert, 1996, Standley *et al.*, 2001; Bramati *et al*., 2002; Bramati *et al*., 2003; Schmandke, 2005). During fermentation, biotic and abiotic stress factors such as wounding, low temperature and pathogen attacks may trigger defenses mechanisms by the synthesis of phenylpropanoid compounds such as flavonoids, isoflavonoids, psoralens, coumarins, phenolic acids, lignin and suberin (Dixon and Paiva, 1995, Rabeta and Lai, 2013). Present result suggested that synthesis of flavonoids in fermented leaves may be similar with the flavonoids content that retained in unfermented leaves and hence led to no differences of TFC between both unfermented and fermented herbal teas.

In present study, there was no significant difference (P > 0.05) of TPC and TFC between both drying techniques in unfermented and fermented *C. nutans* herbal tea. Study by Ji *et al.* (2012) has reported that the TPC of freeze dried sample was comparable with the microwave-vacuum dried samples and more efficient compared to conventional heating such as hot-air drying. During freeze drying, freezing process could lead to the development of ice crystal within the leaves tissues matrix and resulting in greater rupturing cell structure for better solvent accessibility and compounds extraction (Shih *et al.*, 2009). Meanwhile, during microwave-oven drying, the heat generated has more energy efficiency and able to inactive degradative enzymes much faster than conventional techniques. Moreover, the retention of volatile component responsible for flavor was more in microwave-oven drying compared to conventional hot-air drying (Ibrahim *et al*., 2012).

Antioxidant activity of infusions

FRAP assay was used to assess the reduction of Ferric ion-TPTZ complex into ferrous form due to presence of antioxidants in the infusions (Pisoschi and Negulescu, 2011). Based on Table 3, for unfermented *C. nutans* herbal tea, the highest ferric reducing power was recorded in 10 min infusion (438.80 ± 94 mg/L) for freeze dried leaves, while, for fermented *C. nutans* herbal tea, the highest was recorded in 15 min infusion (344.80 ± 66 mg/L) for microwave-oven

Table 3.Reducing power of *Clinacanthus nutans* herbal tea using FRAP assay

Type	Infusion time	Drying technique	
		Freeze Dried	**Microwave-oven dried**
Unfermented *C. nutans* herbal tea[1]	1	403.30 ± 72[a]	350.30 ± 50[a]
	2	360.10 ± 51[a]	366.20 ± 98[a]
	5	377.30 ± 102[a]	378.10 ± 52[a]
	10	438.80 ± 94[a]	416.20 ± 69[a]
	15	373.90 ± 122[a]	383.90 ± 83[a]
	20	396.10 ± 36[a]	396.10 ± 73[a]
Fermented *C. nutans* herbal tea[2]	1	259.80 ± 45[a]	246.20 ± 19[a]
	2	265.70 ± 35[a]	293.80 ± 21[ac]
	5	269.90 ± 16[a]	332.50 ± 45[bce]
	10	273.90 ± 11[a]	296.90 ± 37[ade]
	15	292.80 ± 42[a]	344.80 ± 66[bce]
	20	284.30 ± 28[a]	250.70 ± 49[ad]
BOH Green tea[3]	1	9089.40 ± 272[a]	
	2	9095.60 ± 229[a]	
	5	8564.80 ± 95[b]	
	10	8519.30 ± 203[b]	
	15	8522.90 ± 364[b]	
	20	8475.00 ± 91[b]	
Sabah black tea[4]	1	3753.20 ± 716[a]	
	2	4743.90 ± 589[acd]	
	5	4961.90 ± 681[bcd]	
	10	3976.50 ± 840[ad]	
	15	4713.20 ± 290[ad]	
	20	4766.10 ± 387[ad]	

Mean ± S.D. (n = 5), Same superscripted number or letter within vertical columns are not significantly different (P > 0.05), FRAP values was expressed in ferric reducing ability in 1 liter of infusion (mg/L)

dried leaves. Meanwhile, the free radicals scavenging ability of herbal tea infusion was tested by using free radical ABTS decolorization assay and DPPH radical scavenging assay. In ABTS assay (Table 4), the highest scavenging ability for unfermented C. nutans herbal tea was recorded in 5 min infusion (74.03 ± 2.26 mg AEAC/L) for freeze dried leaves, while, for fermented C. nutans herbal tea, the highest was recorded at 5 min infusion time (62.39 ± 3.96 mg AEAC/L) for microwave-oven dried leaves. In DPPH assay, the inhibition of free radicals activity of *C. nutans* herbal teas was increased in concentration dependent manner (Figure 1.). The unfermented *C. nutans* herbal tea showed higher reducing power and scavenging ability compared to the fermented herbal tea (P < 0.05), however, lower than commercial tea (P < 0.05). This indicated a reduction in antioxidant capacity during tea fermentation (Kim *et al*., 2011). 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. Moreover, there were no significant differences (P > 0.05) of FRAP and ABTS values between both drying techniques. Similar result has also been reported by Chan *et al.* (2009) where microwave-oven drying for 2 min on Etlingera elatior was sufficient to remove moisture content and decompose all heat-labile antioxidants and hence, further drying will not reduce its antioxidant capacity. In freezing process, antioxidants extraction was also sufficient for short period of infusion time, not only due to greater rupture of cell structure but also with the contact with boiling water. The intense heat from boiling water was able to breakdown the cell wall and cellular constituents for efficient antioxidants solubility in boiling water (Amin *et al*., 2006). This suggested that both drying techniques were efficient enough to obtain high antioxidant capacity. However, in terms of drying rate and cost, microwave-oven drying technique is much more preferable for its shorter drying period and affordable for household use.

Relationship between phytochemicals content and antioxidant activities

In present finding of *C. nutans* herbal teas, there was a moderate correlation between the TPC and FRAP values (r = 0.507, P < 0.05) and also ABTS values (r = 0.228, P < 0.05). Supported by Ganguly (2003), phenolics compounds including polyphenols and catechin were reported to be a major group that present in *C. sinensis* tea where the presence of these compounds enables it to scavenge free radicals in human cells. The presence of hydroxyl group on aromatic ring in phenolics compound have the capability to scavenge free radicals via hydrogen or electron transfer (Jing *et al*., 2011). A correlation

Table 4.Scavenging power of *Clinacanthus nutans* herbal tea using ABTS assay

Type	Infusion time	Drying technique	
		Freeze Dried	**Microwave-oven dried**
Unfermented *C. nutans* herbal tea[1]	1	72.49 ± 1.97^{a}	71.87 ± 1.07^{a}
	2	72.99 ± 2.23^{a}	71.88 ± 3.17^{a}
	5	74.03 ± 2.26^{a}	70.69 ± 3.93^{a}
	10	73.93 ± 3.87^{a}	71.65 ± 2.28^{a}
	15	72.41 ± 2.71^{a}	72.87 ± 1.84^{a}
	20	73.43 ± 1.94^{a}	72.82 ± 2.00^{a}
Fermented *C. nutans* herbal tea[2]	1	55.29 ± 4.09^{acd}	61.67 ± 7.53^{ad}
	2	48.23 ± 5.46^{a}	51.99 ± 6.29^{acd}
	5	52.47 ± 3.36^{acd}	62.39 ± 3.96^{ad}
	10	55.01 ± 2.13^{acd}	50.36 ± 4.07^{bce}
	15	56.66 ± 2.45^{bcd}	60.52 ± 5.61^{ae}
	20	57.98 ± 3.55^{bd}	51.55 ± 4.91^{ae}
BOH Green tea[3]	1	71.36 ± 1.31^{a}	
	2	72.42 ± 2.25^{a}	
	5	73.81 ± 2.43^{a}	
	10	75.19 ± 1.94^{a}	
	15	73.45 ± 3.18^{a}	
	20	73.46 ± 2.36^{a}	
Sabah black tea[4]	1	71.87 ± 2.39^{a}	
	2	71.86 ± 2.08^{a}	
	5	71.25 ± 4.10^{a}	
	10	73.25 ± 2.94^{a}	
	15	73.53 ± 2.61^{a}	
	20	73.37 ± 1.72^{a}	

Mean ± S.D. (n = 5), Same superscripted number or letter within vertical columns are not significantly different (P > 0.05), ABTS values was expressed in milligram ascorbic acid equivalent antioxidant capacity in 1 liter of infusion (mg AEAC/L)

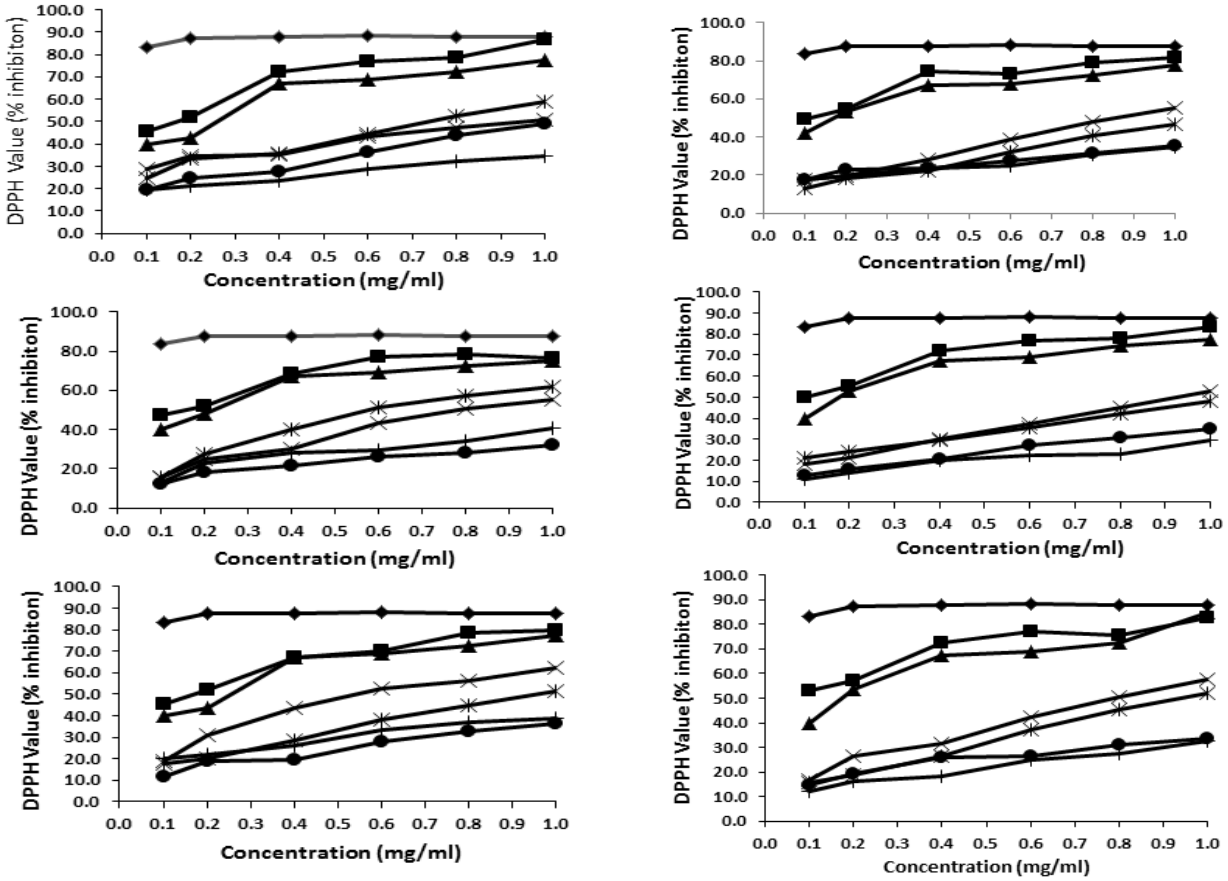

Figure 1.Percentage of inhibition by DPPH by *Clinacanthus nutans* herbal tea with different infusion time; (A) 1 min infusion time (B) 2 min infusion time (C) 5 min infusion time (D) 10 min infusion time (E) 15 min infusion time (F) 20 min infusion time. ♦ Ascorbic acid ■ BOH Green tea ▲SABAH Black tea *C. nutans* herbal tea (UN/FD) *C. nutans* herbal tea (UN/MD) ● *C. nutans* herbal tea (FER/FD) *C. nutans* herbal tea (FER/MD)

between phenolics compounds and antioxidant capacity also been reported by Sabli *et al*. (2012), Ji *et al*., (2012) and Shan *et al*. (2005). However, the TFC in this present study showed a small insignificant correlation with FRAP values ($r = 0.010$, $P > 0.05$) but moderate correlation with ABTS values ($r = 0.256$, $P < 0.05$) assays. According to Wong *et al*., (2013), the aluminum chloride method used in this study basically only specifies the present on flavones and flavonols and not the exact total flavonoids content. Moreover, flavones have been reported to possess no antioxidant ability (Cai *et al*., 2006). This probably has led to insignificant correlation between flavonoid content and antioxidant activity (Meda *et al.*, 2005; Prasad *et al.*, 2009). The insignificant of flavonoids content with antioxidant activity has also been supported by Conforti *et al.* (2009), Andarwulan *et al.* (2010) and Tan *et al.* (2011). Moreover, the antioxidant activity of *C. nutans* herbal tea might also been contributed by the presence of other antioxidants or non-polyphenolic substances such as carotenoids, tocopherol, ascorbic acids and minerals (Ratnam *et al*., 2006).

Conclusion

The preparation and fermentation can influence the TPC, TFC and antioxidant capacity in *C. nutans* herbal tea. Unfermented herbal teas have higher antioxidant capacity than the fermented due to deterioration of phenolics compounds during fermentation. Moreover, *C. nutans* herbal tea preparation only required short infusion time (5 - 15 min) to obtain the same antioxidant capacity as longer infusion time. The microwave-oven drying produced a similar antioxidant capacity as freeze drying, however, in terms of drying rate and cost, microwave-oven drying is preferred than freeze drying for its faster drying rate and affordable for household use. Further studies to determine the sensory quality parameters of this herbal tea, such as color, flavor and fragrance will be carried out. A high performance liquid chromatrography (HPLC) method will also be carried out to determine the phenolics compounds present in infusions. By analyzing *C. nutans* herbal tea, the use especially the Malaysian local herbs in household beverage preparations for health benefits are created. These findings showed that *C. nutans* herbal tea as a potential natural antioxidant source and it can be used as a basic reference for future research on the dietary intake and preparation of these herbal teas.

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