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Effects of Solvent System, Drying and Storage on the Total Phenolic Content and Antioxidant Activities of *Clinacanthus nutans* Lindau (Sabah Snake Grass)

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ABSTRACT

The effects of extraction solvent system (boiling water, water at room temperature, 50% aqueous methanol and 100% methanol), drying (oven drying and sun drying) and storage (refrigeration) on the total phenolic content (TPC) and antioxidant activities of the extracts from *Clinacanthus nutans* Lindau (Sabah Snake Grass) leaves were studied. TPC was determined using Folin-Ciocalteau method. Antioxidant activities were evaluated using three different methods, namely i) 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ii) ferric reducing antioxidant power (FRAP) assay, and iii) beta-carotene bleaching (BCB) assay. Boiling water extraction of fresh leaves resulted in the highest TPC and DPPH and FRAP activities. However, methanolic (100% methanol) extract from the fresh leaves showed the highest antioxidant activity in BCB. Both oven drying and sun drying caused a significant decrease in the antioxidant capacity with sun drying resulting in a lower TPC than oven drying. Cold storage (at 4 ± 2°C) for three weeks after drying resulted in a decrease in TPC. A significant decrease was observed in DPPH, FRAP and BCB activities after three weeks of refrigeration storage. TPC was strongly correlated to DPPH, $1/EC_{50}$ ($r^2 = 0.969$, $p < 0.05$) and FRAP ($r^2 = 0.991$, $p < 0.01$) activities. However, there was no correlation between TPC and BCB. In conclusion, boiling water extraction of fresh *C. nutans* leaves resulted in the highest TPC and antioxidant activities. Drying and storage resulted in deterioration of the TPC and antioxidant activities of *C. nutans* leaves.

Keywords: Antioxidant, *Clinacanthus nutans* Lindau, drying, phenolic content, solvent, storage

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INTRODUCTION

Non-communicable diseases such as cancer, stroke and cardiovascular disease have become a major cause of human mortality. According to World Health Organization [WHO] (2013), an estimated of 36 million deaths (or 63%) of the 57 million deaths globally in 2008 were due to non-communicable diseases. Cancer claims about 7.6 million lives worldwide each year (Union for International Cancer Control [UICC], 2013). One of the causes of non-communicable diseases is the exposure to free radicals. Free radicals are often associated with many diseases including cancer, cataracts, stroke, gastrointestinal disease, and arteriosclerosis (Fu et al., 2011; Greenly, 2004).

Antioxidants are chemical compounds that are able to bind and neutralize free radicals thus preventing the free radicals from causing cell damage (Devasagayam et al., 2004). Antioxidants are important in health care because of their desirable biological effects, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects (Krishnaiah, Sarbatly, & Nithyanandam, 2011). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used in the food industry as a food preservative to prevent the oxidation of lipids that leads to altered taste and smell, especially in high-fat foods (Williams, Iatropoulos, & Whysner, 1999). BHT, however, has been reported to enhance tumour development in the liver, lung and gastro-intestinal tract (Witschi, 1986). This has resulted in consumers concerns on the potential harmful effects of synthetic antioxidants on human health (Maisuthisakul, Suttajit, & Pongsawatmanit, 2007). Natural antioxidants have gained increasing attention and interest because they are regarded as safe for human consumption (Qader et al., 2011). Natural antioxidants are readily available in vegetables, fruits and plants (Fu et al., 2011). Antioxidants from plants have received much attention recently due to their effectiveness in scavenging free radicals. Besides, epidemiological studies have found a close relationship between the intake of certain plant extracts and the reduced risks of chronic diseases such as atherosclerosis and cancer (Podsędek, 2007). This is due to the high content of antioxidant compounds found in certain plants. Certain plants have been used as traditional medicine since ancient times and continue to provide treatment for different diseases (Krishnaiah et al., 2011). *Clinacanthus nutans* Lindau (CN), also known as Sabah snake grass, is a traditional herb from the Acanthaceae family. It is used in Thailand as traditional medicine to treat inflammation, viral infections, herpes infections, snake bites, and varicella zoster virus (Sittiso et al., 2010)

In the present study, the effects of extraction solvent system, drying and storage on the total phenolic content (TPC) and antioxidant activities of the extracts from CN leaves were investigated. Phenolic compounds were extracted from CN leaves using four extraction solvent systems, namely i) boiling water, ii) water at room

temperature, iii) 50% aqueous methanol, and iv) 100% methanol. Three different types of CN leaf samples were studied, namely i) fresh, ii) dried, and iii) stored. Sun drying and oven drying were studied for their drying effects. Subsequent storage study after drying was carried out under refrigeration conditions for three weeks. The TPC and the antioxidant activities of the different extracts obtained were determined.

MATERIALS AND METHODS

Plant Materials

Fresh, harvested *C. nutans* plants were obtained from a local supplier in the Sunday morning market in Gaya Street in Kota Kinabalu, Sabah. The plants were in the maturity stage of around three to ten weeks. The leaves were separated from the plant stems and the selection was based on colour, size and physical appearance to ensure uniformity. Small and discoloured leaves were discarded. Those with white and black spots and holes were also excluded.

Sample Preparation

All fresh leaf samples were briefly cleaned with tap water. For drying study, oven drying and sun drying were carried out. Oven drying was carried out in an oven dryer (Thermoline) for 24 hr at 50 ± 2°C. For sun drying, the fresh leaf sample was placed in a drying rack under the sun for two consecutive days. The sun exposure was 7-9 hr a day depending on the weather conditions. For storage study, the dried sample was kept in a sealed container and stored in a refrigerator (4 ± 2°C) for three weeks. Antioxidant analyses were carried out weekly during the storage period. Moisture contents of the samples were determined to allow all results to be reported in dry weight (DW) basis.

Sample Extraction

Prior to extraction, all leaf samples were homogenized with a food blender (MX-337, Panasonic). Four extraction solvent systems were used, namely boiling water, water at room temperature, 50% (v/v) aqueous methanol, and 100% methanol. For boiling water extraction, the homogenized sample (5 g) was boiled in distilled water (100 mL) at 90-100°C for 10 min. For the other extraction solvent systems, the homogenized sample (5 g) was added with the respective solvent (100 mL) in a conical flask and the mixture was placed in a shaker incubator (WiseCube WIS-20, Witeg) at room temperature for 24 hr.

Determination of Total Phenolic Content (TPC)

The TPC of the plant extracts was determined using Folin-Ciocalteau's reagent according to the method described by Surveswaran, Cai, Corke and Sun (2007) with slight modifications. Plant extract (200 µL; 1 mg/mL for fresh and dried samples, 5 mg/mL for stored samples) and Folin-Ciocalteu (750 µL; 10-fold dilution) were added to a test tube. Higher concentration was used for the stored samples due to their

lower TPC after storage. The mixture was allowed to react for 5 min at room temperature before the addition of 300 µL sodium carbonate (75 g/L) to neutralize the reaction. The mixture was left for 30 min in dark conditions at room temperature to allow complete reaction to form a blue complex. The absorption of the sample was measured at 765 nm against a blank sample using a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer). The TPC of the sample was determined based on the calibration graph (absorbance versus concentration; $y = 0.3135x$, $r^2 = 0.9932$) of gallic acid constructed using 0-5 mg/mL gallic acid. TPC was determined based on the equation below and expressed as mg gallic acid equivalents/100 g dry weight (mg GAE/100 g DW).

$$\text{TPC (mg GAE/100 g DW)} = C \times \left[\frac{V}{m\,(100\% - k)}\right] \times 100000$$

where,

C is the gallic acid concentration obtained from the gallic acid calibration graph (mg/mL),

V is the volume of the extract (mL),
m is the weight of the extract (mg),
k is the moisture content (%).

Determination of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The radical scavenging activity of the plant extracts was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as described by Kchaou, Abbes, Blecker, Attia and Besbes (2013) and Yang, Gadi, Paulino and Thomson (2010). The plant extract (0.5 mL) of 0.5 mg/mL concentration was added to 1.0 mL DPPH (0.1 mM) in methanol. The mixture was kept for 30 min in dark conditions at room temperature. The absorption of the sample was measured against a blank sample at 517 nm with a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer). The procedure was repeated 6-8 times using the same plant extract and the same volume (0.5 mL) but different plant extract concentrations (up to 3.0 mg/mL). This was to obtain a radical scavenging activity (%) curve as a function of plant extract concentration. The radical scavenging activity (%) of each plant extract concentration was determined according to the equation below. Based on the equation of the linear regression line obtained, the EC_{50} of the plant extract was determined. EC_{50} is defined as the concentration of the extract that reduces the initial concentration of DPPH radical initiator by 50%. Ascorbic acid was used as positive control for DPPH analysis. The procedure for the determination of the EC_{50} of ascorbic acid was similar to that of the plant extract except that ascorbic acid was used instead of the plant extract.

$$\text{Radical scavenging activity (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where,

A_o is the absorption of the control sample (blank, without extract),

A_1 is the absorption of the sample extract.

Determination of Ferric Ion Reducing Antioxidant Power (FRAP)

FRAP was determined according to the method described by Bakar, Mohamed, Rahmat and Fry (2009). FRAP reagent was prepared by mixing 300 mM acetate buffer solution at pH 3.6 (adjusted with sodium hydroxide), 10 mM solution of 2,4,6-tripyridyl-s-traizine (TPTZ) in 40 mM hydrochloric acid (HCl) and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) in the volume ratio of 10: 1: 1. The reagent was placed in a water bath for 4 min at 37°C.

FRAP reagent (3 mL) was mixed with 0.1 mL plant extract (1 mg/mL for the fresh and the dried samples; 5 mg/mL for the stored samples) and incubated for 30 min in a water bath (37°C). Absorbance was measured with a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer) at 593 nm. Based on the absorbance, the ferric ion reducing power (FRAP) of the plant extract was determined based on the calibration graph (absorbance versus concentration; $y = 0.5847$, $r^2 = 0.9927$) of ferrous sulphate constructed using 0-2 mmol ferrous sulfate. FRAP was calculated based on the equation below and expressed as mmol ferrous sulfate per 100 g dry weight (mmol $Fe_2SO_4.7H_2O$/100 g DW).
FRAP (mmol Fe2SO4.7H2O/ 100 g DW) =C × [(V)/(m (100%-k))]×100000

where,

C is the concentration of ferrous sulfate obtained from the ferrous sulfate calibration graph (mmol),

V is the volume of the extract (mL),
m is the weight of the extract (mg),
k is the moisture content (%).

Determination of β-Carotene Bleaching (BCB)

BCB assay was carried out according to the method described by Velioglu, Mazza, Gao and Oomah (1998) with some modifications. β-carotene (1 mL) of 0.2 mg/mL in chloroform was added to a 50 mL round bottom flask containing 20 µL linoleic acid and 200 µL Tween 20. The mixture was evaporated using a rotary evaporator (Laborota 400, Heidolph) for 10 min at 40°C under reduced pressure conditions. Thereafter, distilled water (100 mL) was added and the mixture was subjected to vigorous shaking for one min to form an emulsion. The emulsion (5 mL) was added to 200 µL plant extract (1 mg/mL for fresh and dried samples; 5 mg/mL for stored samples) and the mixture was left in dark conditions at 40°C for 120 min. The absorbance of the mixture at 470 nm was measured at t = 0, 20, 40, 60, 80, 100 and 120 min with a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer). Butylated hydroxyanisole, BHA (1 mg/mL in methanol) and methanol were used as standard and negative control, respectively. The amount (%) of inhibition was calculated using the two equations below (Al-Saikhan, Howard, & Miller, 1995).

Rate of bleaching, $R = [\ln (A_0/A_t)]/t$

where,

A_0 = absorbance of the mixture at t = 0 min

A_t = absorbance of the mixture at t = 20, 40, 60, 80, 100 and 120 t = min

$$\text{Inhibition (\%)} = \left[\frac{(R_{control} - R_{sample})}{R_{control}}\right] \times 100$$

where,

$R_{control}$ is the rate of bleaching in the control sample,

R_{sample} is the rate of bleaching in sample extract.

Statistical Analysis

All analyses were conducted in triplicate and the results (in dry weight (DW) basis) are expressed as mean ± standard deviation. Statistical analysis of data was carried out using SPSS (Statistical Package for Social Sciences version 21.0., IBM SPSS Statistics, USA). One-way ANOVA (analysis of variance) was carried out. Comparison of the different extraction solvent systems (i.e. boiling water, water at room temperature, 50% methanol and 100% methanol) and the different types of samples (i.e. fresh, oven-dried and sun-dried) was carried out using Duncan's post hoc multiple comparisons test. Bivariate Pearson's correlation test was used to determine the correlations between the TPC and the antioxidant activities (DPPH, FRAP, BCB). All analyses were carried out at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Solvent System

The TPC and the antioxidant activities (DPPH, FRAP and BCB) of the extracts obtained using different solvent systems from the fresh and the dried leaf samples are shown in Table 1 and Table 2, respectively. For all three samples (fresh, oven-dried and sun-dried), boiling water extraction (474.89 ± 40 to 1453.64 ± 205 mg GAE/100 g DW) resulted in the highest TPC, followed by water at room temperature (300.42 ± 92 to 1040.17 ± 121 mg GAE/100 g DW), 50% methanol (197.68 ± 55 to 807.10 ± 89 mg GAE/100 g DW) and 100% methanol (117.05 ± 19 to 516.51 ± 63 mg GAE/100 g DW) extractions. Water is a very polar solvent. Methanol is also a polar solvent but is less polar than water. The results suggest that most of the phenolic compounds present in all three samples were of high polarity. Boiling water extraction resulted in a higher TPC than water extraction at room temperature because the high temperature of boiling water can liberate the phenolic compounds that are bonded covalently to the cell wall (Lattanzio, Lattanzio, & Cardinali, 2006). Such phenolic compounds may not be extracted by water extraction at room temperature.

Based on the results shown in Table 1 and Table 2, it was noted that high TPC resulted in high DPPH and FRAP activities for all three samples regardless of solvent system. The results suggest all phenolic compounds present in all three samples contribute significantly to DPPH

Table 1
TPC in fresh and dry leaves of Clinacanthus nutans *Lindau*

Solvent system	TPC		
	mg GAE/100 g DW		
	Fresh	Oven drying	Sun drying
Boiling water	1453.64 ± 205^{a1}	967.11 ± 82^{a2} (-33.5%)	474.89 ± 40^{a3} (-67.3%)
Water	1040.17 ± 121^{b1}	719.09 ± 89^{b2} (-30.9%)	300.42 ± 92^{b3} (-71.1%)
50% Methanol	807.10 ± 89^{b1}	567.30 ± 129^{b2} (-29.8%)	197.68 ± 55^{bc3} (-66.2%)
100% Methanol	516.51 ± 63^{c1}	339.03 ± 65^{c2} (-34.4%)	117.05 ± 19^{c3} (-77.4%)

For each column in each assay, values with different letter superscripts (a-d) denote significant differences ($p < 0.05$).
For each row in each assay, values with different number superscripts (1-3) denote significant differences ($p < 0.05$).
Values in parentheses are the changes (%) compared to the fresh sample where the signs '+' and '-' refer to an increase and a decrease, respectively.

and FRAP activities. Although the TPC of the 50% methanol and the 100% methanol extractions were lower, these TPC resulted in a higher BCB antioxidant activity when compared to those obtained through boiling water and water at room temperature extractions regardless of the type of sample. This suggests that these relatively less polar phenolic compounds obtained through 50% methanol and 100% methanol extractions contribute more significantly to BCB activity than those more polar phenolic compounds obtained through aqueous (boiling water and water at room temperature) extractions. The antioxidant mechanism based on hydrogen atom transfer (HAT) of BCB is different from those of DPPH and FRAP which are based on single electron transfer (SET) (Apak et al., 2013). In all three samples, the relatively less polar phenolic compounds seem to be more in favour of the HAT mechanism in their antioxidant activities than those more polar phenolic compounds.

Effect of Drying

For all four solvent systems, fresh sample (516.51 ± 63 to 1453.64 ± 205 mg GAE/100 g DW) had the highest TPC, followed by oven-dried sample (339.03 ± 65 to 967.11 ± 82 mg GAE/100 g DW) and sun-dried sample (117.05 ± 19 to 474.89 ± 40 mg GAE/100 g DW) (Table 1). Both oven drying and sun drying resulted in the reduction of TPC. When compared to fresh sample, sun drying (-66.2 to -77.4%) caused a higher loss of TPC than oven drying

Table 2

DPPH, FRAP and BCB activities in fresh and dry leaves of Clinacanthus nutans *Lindau*

Solvent system	DPPH			FRAP			BCB		
	EC_{50} (mg/mL)			mmol $Fe_2SO_4.7H_2O$/100 g DW			Inhibition (%)		
	Fresh	Oven drying	Sun drying	Fresh	Oven drying	Sun drying	Fresh	Oven drying	Sun drying
Boiling water	0.26 ± 0.05^{a1}	1.10 ± 0.05^{a2} (+323.1%)	1.61 ± 0.17^{a3} (+519.2%)	348.56 ± 32^{a1}	264.59 ± 45^{a2} (-24.1%)	165.15 ± 18^{a3} (-52.6%)	80.83 ± 0.41^{a1}	73.68 ± 0.94^{a2} (-8.8%)	64.60 ± 2.73^{a3} (-20.1%)
Water	0.51 ± 0.15^{b1}	2.07 ± 0.50^{b2} (+305.9%)	2.94 ± 0.24^{b3} (+476.5%)	269.36 ± 42^{b1}	166.30 ± 35^{b2} (-38.3%)	95.91 ± 13^{b3} (-64.4%)	76.38 ± 1.41^{b1}	64.45 ± 2.13^{b2} (-15.6%)	60.89 ± 4.74^{a2} (-20.3%)
50% Methanol	0.87 ± 0.03^{c1}	3.79 ± 0.72^{c2} (+335.6%)	4.73 ± 0.19^{c3} (+443.7%)	185.23 ± 52^{c1}	113.79 ± 18^{b2} (-38.6%)	65.97 ± 25^{b2} (-64.4%)	88.33 ± 0.87^{c1}	82.25 ± 1.53^{c2} (-6.9%)	73.20 ± 2.72^{b3} (-17.1%)
100% Methanol	1.22 ± 0.06^{d1}	4.79 ± 0.60^{d2} (+292.6%)	5.54 ± 0.23^{d3} (+354.1%)	101.27 ± 17^{d1}	53.17 ± 10^{c2} (-47.5%)	23.12 ± 4^{c3} (-77.2%)	92.12 ± 0.71^{d1}	86.87 ± 1.79^{d2} (-5.7%)	81.78 ± 1.23^{c3} (-11.2%)
Standard	Ascorbic acid: 0.0260 ± 0.0001^{e}						Butylated hydroxyanisole (BHA): 98.09 ± 0.39^{e}		

For each column in each assay, values with different letter superscripts (a-d) denote significant differences ($p < 0.05$).

For each row in each assay, values with different number superscripts (1-3) denote significant differences ($p < 0.05$).

Values in parentheses are the changes (%) compared to fresh sample where the signs '+' and '-' refer to an increase and a decrease, respectively.

(-29.8 to -34.4%). Phenolic compounds are thermally degraded due to their high heat sensitivity and susceptibility to oxidation (Lim & Murtijaya, 2007). For both oven drying and sun drying, the TPC obtained through 50% methanol (-29.8% and -66.2%, respectively) had the lowest reduction after drying, followed by boiling water (-33.5% and -67.3%, respectively), water (-30.9% and -71.7%, respectively) and 100% methanol (-34.4% and -77.4%, respectively). This suggests the phenolic compounds extracted with 50% methanol had the highest thermal stability thus experiencing the least thermal degradation during drying.

The loss of TPC after drying may be due to the enzymatic reactions involving polyphenol oxidase during drying (Cavalcanti, Resende, Carvalho, Silveira, & Oliveira, 2006). Polyphenol oxidase acting as an enzyme catalyst in the hydroxylation reaction in the presence of oxygen has the ability to degrade and destroy phenolic compounds. The lower TPC for sun drying may be due to the slower deactivation of the degradative enzyme when compared to oven drying. More phenolic compounds were degraded in sun drying because of the slower drying process due to the uneven heat distribution across the leaf sample and the temperature fluctuation. On the other hand, oven drying involved placing the leaf sample in a preheated oven dryer at 50°C causing thermal shock and eventually deactivation of the enzymes (Jaiswal, DerMarderosian, & Porter, 2010). Besides, oven drying had a more uniform and consistent heat transfer than sun drying causing the heat-labile enzymes to be degraded more rapidly. However, some enzymatic reactions may have taken place in the early stages of the drying process which resulted in some degradation of phenolic compounds (Lim & Murtijaya, 2007). Jaiswal et al. (2010) also reported a higher reduction of anthocyanin (a phenolic compound) in pomegranate in sun drying when compared to oven drying. It was found that a higher deterioration rate of polyphenol oxidase during drying resulted in a lower reduction rate of the anthocyanin. A 61% loss of anthocyanin for oven drying was observed when the polyphenol oxidase deterioration was 68%. When the polyphenol oxidase deterioration was lower at 45% for sun drying, a higher anthocyanin loss at 83% was observed.

The reduction in TPC after drying caused similar effect on the DPPH, FRAP and BCB activities. A decrease in the DPPH antioxidant activity is reflected by an increase in the EC_{50} value. Oven drying resulted in lower FRAP reduction (-24.1 to -47.5%) when compared to sun drying (-52.6 to -77.2%) (Table 2). The polar phenolic compounds obtained through boiling water extraction recorded the lowest reduction in FRAP antioxidant activity. Similar to FRAP, a lower reduction in BCB antioxidant activity was observed for oven drying (-5.7 to -15.6%) when compared to sun drying (-11.2 to -20.3%). In contrast to FRAP, the relatively less polar phenolic compounds obtained through 50% methanol and 100% methanol extractions had a lower reduction in BCB antioxidant activity.

Effect of Storage

The TPC and the antioxidant activities (DPPH, FRAP and BCB) of the extracts from the dried leaf samples during the 3-week cold storage period are shown in Table 3 and Table 4, respectively. For both oven drying and sun drying, the TPC and the antioxidant activities decreased with storage time regardless of the solvent system. Boiling water extraction resulted in the highest TPC regardless of the storage time (Table 3). Similar to the fresh sample (Table 1), the amount of phenolic compounds extracted by the four solvent systems from the dried samples (both oven drying and sun drying) was in the order of boiling water > water at room temperature > 50% methanol > 100% methanol. A similar order was observed for the stored samples regardless of the storage time. The reduction in phenolic compounds after one, two and three weeks of storage was -4.0 to -12.2%, -6.6 to -14.1%, and -11.3 to -29.2%, respectively, depending on the solvent system. For all four solvent systems, the reduction of phenolic compounds increased with increasing storage time. Upon cold storage for three weeks, the total reduction in phenolic compounds in the oven-dried sample and the sun-dried sample was -11.3 to -25.5% and -19.1 to -29.2%, respectively, depending on the solvent system. The rates of reduction in phenolic compounds due to storage (-11.3 to -29.2%) (Table 3) were lower than those caused by drying (-29.8 to -77.4%) (Table 1). This may be due to the phenolic compounds of high susceptibility to oxidation being degraded during the drying process. The residual phenolic compounds after the drying process were those of higher stability and therefore experienced less oxidation during the storage (Srivastava, 2006). In addition, the low temperature during cold storage presented less oxidative stress when compared to the high temperature applied during drying. For both oven drying and sun drying, the final reduction rate of phenolic compounds at the end of the 3-week storage decreased with increasing polarity of the solvent (Table 3). This indicates the more polar phenolic compounds (obtained through more polar solvents) were more stable and therefore experienced less degradation during storage when compared to the relatively less polar compounds (obtained through relatively less polar solvents). According to Kevers et al. (2007), the stability of phenolic compounds in plants during storage varies greatly depending on the type of plant and the type of polyphenol compounds. The TPC in broccoli was found to increase during the early stages of 27-day cold-storage before declining sharply. For 8-day cold storage, the decrease in TPC was 30% on the second day for lettuce and 50% on the fifth day for celery. The decrease of the TPC in both vegetables may be caused by the deterioration of flavonoids, a group of polyphenol compounds.

For both oven drying and sun drying, DPPH, FRAP and BCB activities decreased with increasing storage time regardless of

Table 3

TPC in dry leaves of Clinacanthus nutans *Lindau after storage*

Solvent system	TPC		
	mg GAE/100 g DW		
	Week 1	Week 2	Week 3
Boiling water (Oven drying)	973.33 ± 16^{a12} (-4.0%)	953.56 ± 17^{a2} (-6.0%)	899.16 ± 11^{a3} (-11.3%)
	Week 0: 1013.99 ± 11^{a1}		
Water (Oven drying)	728.01 ± 20^{b12} (-5.4%)	711.67 ± 21^{b2} (-7.5%)	677.47 ± 34^{b3} (-11.9%)
	Week 0: 769.22 ± 27^{b1}		
50% Methanol (Oven drying)	468.69 ± 23^{c12} (-5.0%)	454.93 ± 22^{c2} (-7.8%)	394.07 ± 20^{c3} (-20.1%)
	Week 0: 493.41 ± 26^{c1}		
100% Methanol (Oven drying)	354.42 ± 26^{d12} (-5.4%)	335.86 ± 24^{d2} (-10.4%)	274.84 ± 16^{d3} (-25.6%)
	Week 0: 374.77 ± 30^{d1}		
Boiling water (Sun drying)	466.63 ± 14^{c12} (-6.1%)	453.55 ± 11^{c2} (-8.7%)	401.84 ± 11^{c3} (-19.1%)
	Week 0: 496.94 ± 16^{c1}		
Water (Sun drying)	320.31 ± 72^{d1} (-9.43%)	306.58 ± 73^{d2} (-10.5%)	268.24 ± 63^{d3} (-21.8%)
	Week 0: 342.67 ± 79^{d1}		
50% Methanol (Sun drying)	194.23 ± 53^{e1} (-11.6%)	188.71 ± 51^{e12} (-14.1%)	157.71 ± 40^{e2} (-28.2%)
	Week 0: 219.74 ± 57^{e1}		
100% Methanol (Sun drying)	111.61 ± 10^{f12} (-12.2%)	102.47 ± 10^{f2} (-19.4%)	90.00 ± 10^{e3} (-29.2%)
	Week 0: 127.08 ± 11^{f1}		

For each column in each assay, values with different letter superscripts (a-f) denote significant differences ($p < 0.05$).

For each row in each assay, values with different number superscripts (1-3) denote significant differences ($p < 0.05$).

Values in parentheses are the changes (%) compared to Week 0 where the signs '+' and '-' refer to an ncrease and a decrease, respectively.

Table 4

DPPH, FRAP and BCB activities in dry leaves of Clinacanthus nutans *Lindau after storage*

Solvent system	DPPH			FRAP			BCB		
	EC_{50} (mg/mL)			mmol $Fe_2SO_4.7H_2O$/100 g DW			Inhibition (%)		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Boiling water (Oven drying)	1.12 ± 0.04^{a12} (+4.7%)	1.20 ± 0.40^{a23} (+12.1%)	1.28 ± 0.04^{a3} (+19.6%)	228.59 ± 20^{a12} (-4.9%)	216.89 ± 15^{a2} (-9.7%)	182.11 ± 12^{a3} (-24.2%)	69.33 ± 1.06^{a2} (-6.6%)	61.87 ± 0.47^{a3} (-16.6%)	55.67 ± 0.50^{a4} (-25.0%)
	Week 0: 1.07 ± 0.02^{a1}			Week 0: 240.29 ± 22^{a1}			Week 0: 74.21 ± 0.36^{a1}		
Water (Oven drying)	1.92 ± 0.10^{b12} (+7.3%)	2.07 ± 0.11^{b2} (+15.6%)	2.24 ± 0.15^{b2} (+25.1%)	137.09 ± 11^{b12} (-6.5%)	131.10 ± 12^{b2} (-10.6%)	110.34 ± 10^{b3} (-24.7%)	61.48 ± 0.20^{b2} (-6.4%)	55.37 ± 0.53^{b3} (-15.7%)	49.73 ± 1.04^{b4} (-24.3%)
	Week 0: 1.79 ± 0.08^{b1}			Week 0: 146.61 ± 12^{b1}			Week 0: 65.67 ± 0.53^{b1}		
50% Methanol (Oven drying)	3.71 ± 0.24^{c12} (+9.4%)	4.07 ± 0.26^{c2} (+20.1%)	4.45 ± 0.29^{c2} (+31.3%)	98.29 ± 16^{c1} (-7.1%)	93.56 ± 16^{c2} (-11.6%)	75.97 ± 12^{c3} (-25.4%)	78.34 ± 2.01^{c1} (-5.6%)	71.96 ± 1.90^{c2} (-13.3%)	63.75 ± 2.36^{c3} (-23.2%)
	Week 0: 3.39 ± 0.27^{c1}			Week 0: 105.83 ± 17^{c1}			Week 0: 82.98 ± 1.20^{c1}		

Table 4 *(Continue)*

Table 4

DPPH, FRAP and BCB activities in dry leaves of Clinacanthus nutans *Lindau after storage*

Solvent system	DPPH			FRAP			BCB		
	EC_{50} (mg/mL)			mmol $Fe_2SO_4.7H_2O$/100 g DW			Inhibition (%)		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
100% Methanol (Oven drying)	5.15 ± 0.85^{d12} (+12.0%)	5.74 ± 0.93^{d23} (+24.8%)	6.28 ± 1.05^{d3} (+36.5%)	44.75 ± 8^{d12} (-8.3%)	42.80 ± 8^{d2} (-12.3%)	33.91 ± 6^{d2} (-30.5%)	83.89 ± 2.31^{d1} (-4.4%)	76.92 ± 3.18^{d2} (-12.3%)	70.76 ± 2.64^{d2} (-19.3%)
	Week 0: 4.60 ± 0.70^{d1}			Week 0: 48.78 ± 9^{d1}			Week 0: 87.72 ± 1.44^{d1}		
Boiling water (Sun drying)	1.59 ± 0.09^{b12} (+4.6%)	1.70 ± 0.12^{b23} (+11.8%)	1.88 ± 0.11^{b3} (+23.7%)	164.94 ± 13^{b12} (-5.0%)	160.13 ± 11^{b2} (-7.8%)	128.94 ± 11^{b3} (-25.7%)	59.14 ± 0.32^{b2} (-10.6%)	54.34 ± 0.41^{b3} (-17.9%)	48.91 ± 0.95^{b4} (-26.1%)
	Week 0: 1.52 ± 0.08^{b1}			Week 0: 173.63 ± 14^{b1}			Week 0: 66.15 ± 0.69^{b1}		
Water (Sun drying)	3.29 ± 0.27^{c12} (+8.6%)	3.53 ± 0.26^{c2} (+16.5%)	3.82 ± 0.26^{c2} (+26.1%)	91.60 ± 11^{c12} (-9.4%)	82.04 ± 12^{c2} (-18.9%)	71.50 ± 10^{c3} (-29.3%)	57.26 ± 1.57^{b2} (-9.8%)	53.20 ± 1.43^{b2} (-16.2%)	48.42 ± 0.60^{b3} (-23.7%)
	Week 0: 3.03 ± 0.26^{c1}			Week 0: 101.14 ± 13^{c1}			Week 0: 63.46 ± 2.29^{b1}		

Table 4 *(Continue)*

Solvent system	DPPH			FRAP			BCB		
	EC_{50} (mg/mL)			mmol $Fe_2SO_4.7H_2O$/100 g DW			Inhibition (%)		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
50% Methanol (Sun drying)	5.11 ± 0.18^{d12} (+9.7%)	5.65 ± 0.18^{d23} (+21.2%)	6.16 ± 0.25^{d3} (+32.2%)	68.66 ± 14^{d12} (-11.9%)	61.81 ± 12^{d2} (-20.7%)	48.66 ± 10^{d3} (-37.6%)	67.94 ± 3.07^{a12} (-6.9%)	62.07 ± 3.01^{a23} (-14.9%)	57.34 ± 2.22^{a3} (-21.4%)
	Week 0: 4.66 ± 0.21^{d1}			Week 0: 77.92 ± 19^{d1}			Week 0: 72.94 ± 3.79^{a1}		
100% Methanol (Sun drying)	6.21 ± 0.24^{e12} (+11.1%)	7.00 ± 0.35^{e23} (+25.2%)	7.76 ± 0.33^{e3} (+38.8%)	21.79 ± 2^{e12} (-13.1%)	19.44 ± 3^{e2} (-22.5%)	16.10 ± 2^{e2} (-35.8%)	77.89 ± 2.12^{c1} (-5.0%)	70.66 ± 1.78^{c2} (-13.8%)	65.65 ± 2.04^{c2} (-19.9%)
	Week 0: 5.59 ± 0.30^{e1}			Week 0: 25.08 ± 3^{e1}			Week 0: 81.95 ± 1.69^{c1}		
Standard	Ascorbic acid: 0.026 ± 0.0001^{f}						Butylated hydroxyanisole (BHA): 98.09 ± 0.39^{e}		

For each column in each assay, values with different letter superscripts (a-f) denote significant differences ($p < 0.05$).

For each row in each assay, values with different number superscripts (1-3) denote significant differences ($p < 0.05$).

Values in parentheses are the changes (%) compared to Week 0 where the signs '+' and '-' refer to an increase and a decrease, respectively.

solvent system (Table 4). The reduction in the antioxidant activities may be explained by the reduction in TPC. The total reduction of FRAP and BCB activities at the end of the 3-week storage was -24.2 to -37.6% and -19.3 to -26.1%, respectively, depending on the solvent system. The EC_{50} of DPPH increased (indicating a decrease in DPPH activity) by 19.6-38.8% after 3 weeks of storage depending on the solvent system.

Correlation

The total phenolic content extracted from the fresh leaf sample was positively correlated with DPPH activity, $1/EC_{50}$ ($r^2 = 0.969$, $p < 0.05$) and FRAP activity ($r^2 = 0.991$, $p < 0.01$). The antioxidant activities of DPPH and FRAP are based on the single electron transfer (SET) mechanism. SET-based mechanism measures the ability of the antioxidant in donating electrons to reduce (stabilize) the free radicals (Prior, Wu, & Schaich, 2005). The high positive r^2 values (close to 1) suggest the phenolic compounds extracted contribute significantly to DPPH and FRAP activities. This may indicate that most of the phenolic compounds favour the SET mechanism in their antioxidant activities. However, there was no significant correlation between TPC and BCB activity ($r^2 = -0.760$, $p > 0.05$). The BCB antioxidant activity is based on the hydrogen atom transfer (HAT) mechanism. HAT-based mechanism measures the antioxidant capability in preventing free radical chain activities by donating a hydrogen atom (Apak et al., 2007). The lack of correlation between TPC and BCB activity may suggest that most of the phenolic compounds extracted do not proceed through the HAT mechanism in their antioxidant reactions.

CONCLUSION

The extracts obtained from fresh leaf sample of *Clinacanthus nutans* Lindau had the highest TPC and antioxidant activities (DPPH, FRAP and BCB) when compared to the extracts from dried leaf samples. Drying (oven drying and sun drying) reduced the TPC and antioxidant activities with sun drying causing higher reduction effect. Cold storage for three weeks also resulted in a reduction in TPC and antioxidant activities with increasing reduction level with increasing storage time. In term of extraction of TPC, boiling water recorded the highest yield, followed by water at room temperature, 50% methanol and 100% methanol. The TPC of the fresh leaf sample was positively correlated with the DPPH and FRAP activities. No significant correlation was found between the TPC and the BCB activity.

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