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Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower

Yan, S.W. and *Asmah, R.

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor, Malaysia

Abstract: Synthetic antioxidants are added to food in the powdered form to preserve it. However these compounds posed serious health concern since they have been associated with causing cancer. Thus using fresh herbs with antioxidant activities would be good alternative. The objectives of this study were to evaluate and compare the total phenolic contents and antioxidant activities of both powdered and fresh forms of turmeric leaf, pandan leaf and torch ginger flower. Total phenolic content (TPC) was assayed based on the redox reaction between Folin-Ciocalteu with phenolics in the sample extracts. Antioxidant activity (AA) was assayed using the ß-carotene linoleate model system and the percentage of antioxidant activity was calculated from the values of degradation rate. Scavenging activity (SA) was assayed using the DPPH radical scavenging model system whereby EC50 value was determined from the plotted graph of scavenging activity against the concentration of sample extracts. Analyses revealed that powdered forms of turmeric leaf, pandan leaf and torch ginger flower had higher TPC (2013.09 ± 5.13, 1784.25 ± 7.59 and 1937.42 ± 6.61 mg GAE/100g, respectively) than their respective fresh forms (348.75 ± 1.26, 356.42 ± 1.32 and 211.59 ± 6.29 mg GAE/100g, respectively). Similarly, powdered forms of turmeric leaf, pandan leaf and torch ginger flower possessed better AA (64.31 ± 0.99, 65.09 ± 0.74 and 11.80 ± 0.40 %, respectively) than their respective fresh forms (24.93 ± 0.71, 16.91 ± 0.70 and 1.45 ± 0.10 %, respectively). Powdered forms of turmeric leaf, pandan leaf and torch ginger flower were also better radical scavenger as compared to their respective fresh forms. In conclusion, all samples in their powdered forms have high total phenolic contents, antioxidant and scavenging activities than their respective fresh forms.

Key words: Turmeric leaf, pandan leaf, torch ginger flower, antioxidant activity, total phenolic content

Introduction

Turmeric (*Curcuma longa*), pandan (*Pandanus amaryllifolius*) and torch ginger (*Etlingera elatior*) are some of the herbs and spices widely used in South East Asian cooking. Djeridane *et al*. (2006) pointed out that since ancient; herbs have been the principle for virtually all-medicinal therapy until synthetic drugs were developed in the nineteenth century. This view was supported by Hirasa and Takemasa (1998) who mentioned that the protective effect of many plant herbs and spices implies the presence of antioxidative and antimicrobial components in their tissues.

Meanwhile Chen *et al*. (2007) drew our attention to the several etiologies of non-communicable diseases which are of increasing interest to public health. In their review, they reported that reactive oxygen species and free radicals are continuously produced in the human body by normal metabolic action. These have been associated with the pathogenesis of certain human diseases, such as cancer, aging, diabetes and atherosclerosis. Moreover, reactive oxygen species and free radicals are abundantly reactive and basically very unstable (Khaled *et al*., 2007).

Fortunately, antioxidants are capable to prolong or prevent the oxidation of other molecules thereby slowing the progression of those chronic diseases. They act by inhibiting the initiation or proliferation of oxidizing chain reactions (Velioglu *et al*., 1998). It is worthwhile to know that oxidants and antioxidants in humans are sustained in equilibrium in a healthy physiological state. However, aggravating this balance and overproduction of oxidants in numerous circumstances such as smoking, hazardous environmental exposures or infectious diseases can generate oxidative stress resulting in oxidative damage to biomolecules and cells (Suresh *et al*., 2006) as well as cell injury and death (Chen *et al*., 2007).

In recent years, there has been increasing amount of literature in antioxidants. Polyphenols are generally found in both edible and inedible plants and have been reported to have various biological effects including antioxidant activity (Wojdylo *et al*., 2007). They have been known to act as antioxidants

Corresponding author.
Email: *asmah@medic.upm.edu.my*
Tel. +603 89472470

due to their capability to donate electrons as well as the effectiveness of stablilizing radical intermediates in the prevention of oxidation at cellular and physiological level (Suresh *et al*., 2006).

Likewise, epidemiological studies also have shown that consumption of food and beverages with ample phenolic compounds can minimize the risk of heart disease. These compounds diminish the development of atherosclerosis through acting as antioxidants towards low-density lipoprotein (Kaur and Kapoor, 2002).

Noting the abundant beneficial effects of antioxidants to human health, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are often added to food. Nevertheless, this practice is being restrained due to their carcinogenicity and because synthetic antioxidants are known to prompt liver and kidney dysfunction (Velioglu *et al*., 1998). Hence, interest in discovering naturally occurring antioxidants for application in foods or medicinal materials to replace synthetic antioxidants such as BHA and BHT has intensified remarkably.

Therefore, the focus of this study was on turmeric leaf, pandan leaf and torch ginger flower, whereby total phenolic content and antioxidant activity as well as their correlation were determined. These herbs and spices are commonly consumed in Malaysia not only because of their taste, which adds variety and flavour to the diets, but also because of their health benefits.

Most herbs and spices grow well in tropical South East Asia countries, and thus people from these countries can use herbs in the fresh form. However, in countries where these herbs cannot grow due to climatic condition, they have to depend on the powdered form for use. As a consequence, it is important to study the total phenolic content and antioxidant activity of the powdered forms to evaluate whether they differ from that of their fresh forms. It would be beneficial for the food industry to know whether processing of herbs and spices to their powdered forms would cause changes to their chemical constituents or not.

Materials and Methods

Samples

Samples were purchased fresh from Pasar Tani FAMA, Serdang; turmeric leaves (*Cucurma longa*), pandan leaves (*Pandanus amaryllifolius*) and torch ginger flowers (*Etlingera elatior*).

Reagents and chemicals

All chemicals and reagents used were of analytical grade; absolute methanol, Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany); chloroform were from Fischer Scientific (Loughborough, UK); gallic acid, β-carotene, linoleic acid, Tween 20, butylated hydroxytoulene (BHT), ascorbic acid, Tris-HCl buffer (pH 7.4) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma Chemical Co.(St Louis, MO, USA).

Sample preparation

Following purchase, fresh samples were cleaned and washed with excess fresh water. Edible portions (100 g) were cut into small pieces and homogenized using a blender for 2 minutes. The homogenized sample was transferred into air-tight container and kept at –20°C before extraction. These were hereafter referred to as fresh form. For powdered form, the homogenized sample was then kept in the freezer at –80°C overnight, freeze-dried for 3 days, grounded using a dry grinder and fine powders were obtained using a fine mesh sieve and stored at –20°C.

Sample extraction

Two grams of fresh / powdered samples were transferred into a 50 ml volumetric flask and 80% (v/v) methanol were added up to the mark. Mixture was shaken at 200 rpm for 120 minutes at 50°C. The mixture was then centrifuged at 3000 rpm for 15 minutes at room temperature and supernatant was saved. This supernatant was used for total phenolic content, β-carotene bleaching and DPPH radical scavenging assays. All tests were performed within a week. The extracts were stored at –20°C.

Determination of total phenolic content

Determination of total phenolic content was carried out according to Lister and Wilson (2001). One hundred microliters of supernatant extract was dissolved in 1500 µl (1/10 dilution) of the Folin–Ciocalteu reagent. The solutions were mixed and incubated at room temperature for 1 minute.

After 1 minute, 1500 µl of 60g/L sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 1 ½ hour in the dark at room temperature. The absorbance of all samples was measured at 725 nm using UV-Vis spectrophotometer.

Gallic acid was used as standard for the calibration curve and was plotted at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml gallic acid that was prepared in 80% (v/v) methanol. The absorbance was recorded at 725 nm using 80% (v/v) methanol as blank. Triplicate measurements were carried out and total phenolic

content was expressed as milligram of gallic acid equivalents (GAE) per 100 gram of samples.

Determination of antioxidant activity

Determination of antioxidant activity by ß-carotene bleaching method was carried out according to Velioglu *et al*. (1998). One milliliter of ß-carotene solution (0.2 mg/ml in chloroform) was added to a 50 ml round-bottom flask containing 20 µl of linoleic acid and 200 µl of Tween 20. Each mixture was then dosed with 200 µl of samples. For control, samples were replaced by 200 µl 80% (v/v) methanol whereas for standard, samples were replaced by 200 µl 40 g/l BHT.

After evaporation to dryness under vacuum at 40°C for 30 minutes, 100 ml of distilled water was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autoxidation using water bath at 45°C. The absorbance of the solution at 470 nm was monitored on a UV-Vis spectrophotometer by taking measurements at 20 minutes intervals for 120 minutes in dark room to avoid ß-carotene oxidation. Triplicate measurements were carried out and antioxidant activity (AA) was calculated as percent inhibition relative to control using the following equation:

$$\text{AA (\%)} = \frac{R\ control - R\ sample}{R\ control} \times 100$$

Whereby,
R Control = Bleaching rates of ß-carotene in reactant mix without samples.
R Sample = Bleaching rates of ß-carotene in reactant mix with samples.

$$R = \frac{\ln(\frac{A_o}{A_t})}{t}$$

Whereby,
Ao = Absorbance at t = 0 min
At = Absorbance at t = 120 min

Determination of scavenging activity

Determination of scavenging activity by DPPH radical scavenging method was carried out according to Azizah *et al*. (2007). Two hundred microliters of sample extract (0.62 – 4.96 mg/ml in 80% (v/v) methanol) or ascorbic acid (standard) (0.16 – 1.28 mg/ml) was mixed with 800 µl 100 mM Tris–HCl buffer (pH 7.4). Then 1 ml of 500 µM DPPH previously prepared in 80% (v/v) methanol was added. The mixture was shaken vigorously and left to stand for 20 minutes at room temperature in a dark room. Absorbance was read using a UV-Vis spectrophotometer at 517 nm. The scavenging effect on the DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{Absorbance\ of\ sample\ at\ 517nm}{Absorbance\ of\ control\ at\ 517nm} \times 100\right)$$

EC_{50} value was determined from the plotted graph of scavenging activity against the concentration of sample extracts, which was defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out, and their scavenging effect was calculated based on the percentage of DPPH scavenged.

Design and analysis

Data were presented as means ± standard deviation. Statistical analysis was done using Statistical Packages for Social Sciences (SPSS) version 14.0. One-way ANOVA was used to test whether there was a significant difference in total phenolic content, antioxidant activity and scavenging activity between fresh and powdered forms. A significant difference was considered at the level of $p < 0.05$.

Pearson's Correlation Coefficient was used to determine whether there was correlation between total phenolic content, antioxidant activity and scavenging activity between fresh and powdered forms. A significant correlation was considered at the level of $*p < 0.05$ and $**p < 0.01$.

Results and Discussion

Total phenolic content (TPC)

The total phenolic content (TPC) values summarized in Table 1 were quantified based on the linear equation obtained from gallic acid standard calibration curve. Thus, TPC values were expressed as gallic acid equivalent (mg GAE/100 g samples). Comparison of TPC between fresh and powdered forms of turmeric leaf, pandan leaf and torch ginger flower was presented in Figure 1. Results of ANOVA analysis indicated that there was significant difference ($p < 0.05$) between fresh and powdered forms in all samples studied.

Freeze-dried turmeric leaf had the highest TPC (2013.09 mg GAE/100 g powdered form) compared to its fresh forms (348.75 mg GAE/100 g fresh form). Similar trend was observed in torch ginger flower

Table 1. Total phenolic content of samples in fresh and powdered forms of turmeric, pandan and torch ginger flower assayed by Folin-Ciocalteu method.

	Samples	Total Phenolic Content (mg GAE/100 g)
Fresh	Turmeric Leaf	348.75 ± 1.26[a]
	Pandan Leaf	356.42 ± 1.32[a]
	Torch Ginger Flower	211.59 ± 6.29[b]
Powder	Turmeric Leaf	2013.09 ± 5.13[c]
	Pandan Leaf	1784.25 ± 7.59[d]
	Torch Ginger Flower	1937.42 ± 6.61[e]

Values were expressed as mean ± standard deviation (n = 3). Means with different letters were significantly different at the level of $p < 0.05$.

Table 2. Antioxidant activity (%) of samples in fresh and powdered forms of turmeric, pandan and torch ginger flower assayed by ß-carotene bleaching method.

	Samples	Antioxidant Activity (%)
Fresh	Turmeric Leaf	24.93 ± 0.71[a]
	Pandan Leaf	16.91 ± 0.70[b]
	Torch Ginger Flower	1.45 ± 0.10[c]
Powder	Turmeric Leaf	64.31 ± 0.99[d]
	Pandan Leaf	65.09 ± 0.74[d]
	Torch Ginger Flower	11.80 ± 0.40[e]

Values were expressed as mean ± standard deviation (n = 3). Means with different letters were significantly different at the level of $p < 0.05$.

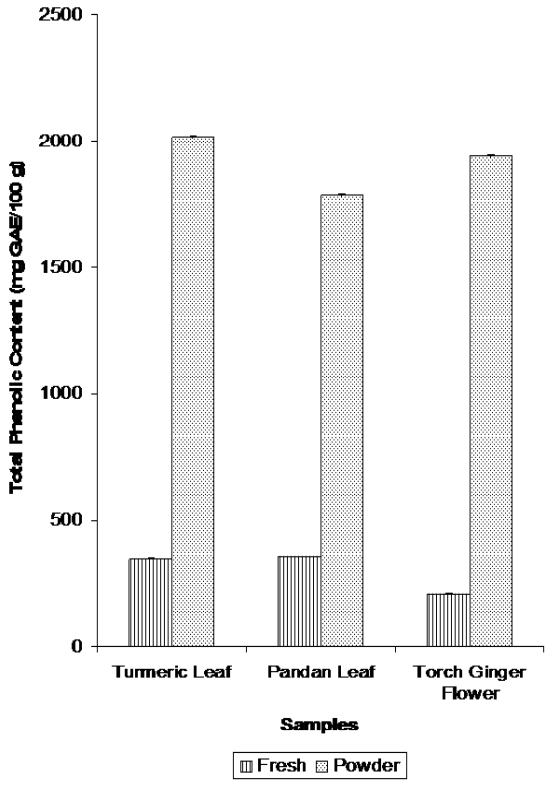

Figure 1. Comparison of total phenolic content between fresh and powdered forms of turmeric, pandan and torch ginger flower assayed by Folin-Ciocalteu method. Means with different letters were significantly different at the level of $p < 0.05$.

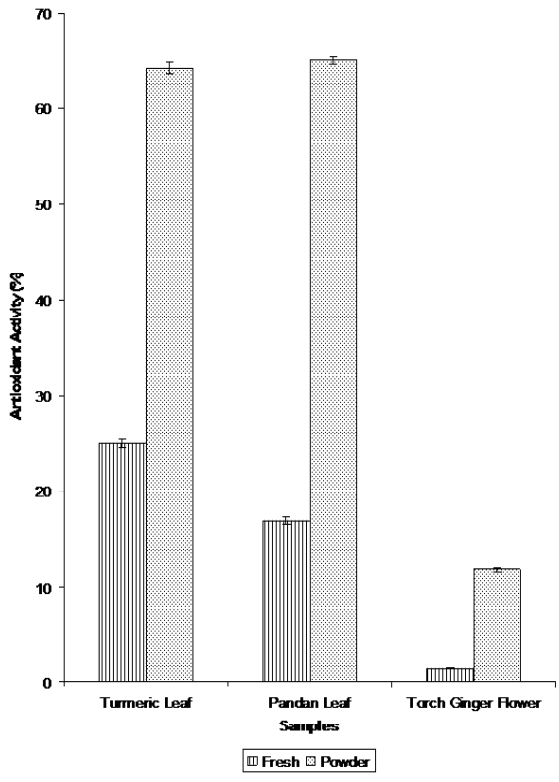

Figure 2. Comparison of antioxidant activity (%) between fresh and powdered forms of turmeric, pandan and torch ginger flower assayed by ß-carotene bleaching method. Means with different letters were significantly different at the level of $p < 0.05$.

whereby TPC of its powdered forms was also higher (1937 mg GAE/100 g powdered form) than its fresh forms (211.59 mg GAE/100 g fresh form). Likewise, TPC in powdered forms of pandan leaf (1784 mg GAE/100 g powdered form) was higher than its fresh forms (356 mg GAE/100 g fresh form). Therefore, results from this assay revealed that all samples in their powdered forms had higher TPC as compared with their respective fresh forms.

This finding was in agreement with the study of Chang *et al*. (2006) whereby they reported that there was a significant increase of TPC in tomatoes through freeze-drying process as compared with its fresh forms which was probably due to the discharge of phenolic compounds from the disintegration of cellular constituents after undergoing freeze-drying process. Although disruption of cell walls might stimulate the release of oxidative and hydrolytic enzymes that would diminish the antioxidants in samples, however Chang *et al*. (2006) reported that at very low temperature freeze-drying would deactivate these enzymes and prevent the loss of phenolic compound, thereby lead to the increase of TPC.

Apart from that, non-phenolic substances such as water, fat, sugars, proteins and pigments may interrupt the evaluation of TPC. Therefore, Djeridane *et al*. (2006) reported that upon extraction procedure to remove these compounds, significant high amount of TPC was obtained in the medicinal plants studied. This may explain for the significantly higher TPC of powdered forms as compared with fresh forms in the present study.

Antioxidant activity (AA)

In this study, butylated hydroxytoluene (BHT) was used as a standard whereas 80% (v/v) methanol, which contained no antioxidant components, was used as a control for comparison with samples extracts for antioxidant activity (AA) assay.

The AA values were summarized in Table 2 and comparison of AA between fresh and powdered forms of turmeric leaf, pandan leaf and torch ginger flower was presented in Figure 2. All samples had lower AA as compared to BHT. Results of ANOVA analysis indicated that there was significant difference ($p < 0.05$) between fresh and powdered forms in all samples.

Freeze-dried pandan leaf had the highest AA (65.09%) as compared to its fresh forms (16.91%). Similarly, freeze-dried turmeric leaf had higher AA (64.31 %) compared to its fresh forms (24.93%). Likewise, AA of freeze-dried torch ginger flower was higher (11.80%) than its fresh forms (1.45%). Therefore, results from this assay revealed that all samples in their powdered forms had higher AA as compared with their respective fresh forms.

Chan *et al*. (2007) reported that fresh leaf of torch ginger had low values in terms of ß-carotene bleaching activity. Similarly, this study found that fresh torch ginger flower had low AA. However, after freeze-dried, AA of torch ginger flower increased the most, with 87.71% increment as compared with its fresh forms.

In another study by Faridah *et al*. (2006), dried forms of pandan leaf were found to be inactive in terms of antioxidant activity. In contrast, this study found that powdered forms of pandan leaf had the highest AA as compared with all other samples, with an increment of 74.02% of activity from its fresh forms. This might be due to different methods applied in quantifying antioxidant activity in both studies, whereby Faridah *et al*. (2006) used ferric thiocyanate (FTC) method while the present study used ß-carotene bleaching method.

We also found that powdered forms of turmeric leaf had relatively high AA, with an increment of 61.23% of activity from that of its fresh forms. It was in agreement with a study by Wojdylo *et al*. (2007) whereby they found that dried rhizomes of C*urcuma longa* had good (62.6 ± 1.01 µM trolox/100 g dry weight) antioxidant capacity.

Statistical analysis showed a positive and moderate ($r = 0.648$) significant correlation between TPC and AA. The finding in this study was in agreement with study by Velioglu *et al*. (1998), whereby they demonstrated a linear relationship between antioxidant capacity and total phenolic in plant products. Besides, Kaur and Kapoor (2002) also reported positive and highly significant relationship between TPC and AA.

Several studies had been made concerning the relationship between the phenolic structure and antioxidant activity but no relationship had been demonstrated because of the many different evaluation systems used to test for antioxidant activity (Kaur and Kapoor, 2002). Besides, Heinonen *et al*. (1998) reported that different phenolic compounds have different responses in the Folin-Ciocalteu method.

Thus, antioxidant activity of samples in this study could not be explained just on the basis of their phenolic content but additional assay had to be conducted to further determine antioxidant activity of samples studied. Therefore, correlation between TPC and SA as well as AA and SA was carried out.

Scavenging activity (SA)

In the present study, ascorbic acid was used as a standard at various concentrations (0.16, 0.32, 0.64,

Table 3. EC_{50} values of samples in fresh and powdered forms of turmeric, pandan and torch ginger flower assayed by DPPH radical scavenging method.

	Samples	EC_{50} (DPPH) (mg/ml)
Fresh	Turmeric Leaf	ND
	Pandan Leaf	ND
	Torch Ginger Flower	ND
Powder	Turmeric Leaf	0.51 ± 0.02^{a}
	Pandan Leaf	0.44 ± 0.01^{b}
	Torch Ginger Flower	0.46 ± 0.01^{b}

Values were expressed as mean ± standard deviation (n = 3).
Means with different letters were significantly different at the level of $p < 0.05$. ND = not detected.

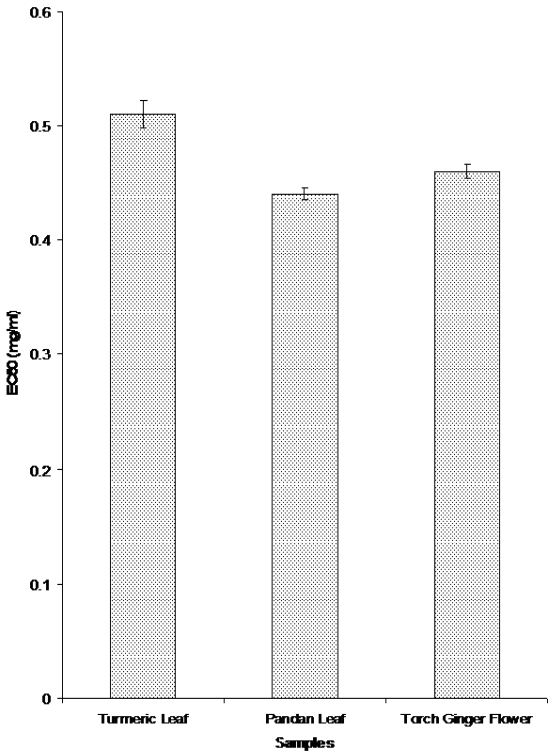

Figure 3. EC_{50} values (extract concentration with 50 % of maximum) of powdered forms of turmeric, pandan and torch ginger flower assayed by DPPH radical scavenging method. Means with different letters were significantly different at the level of $p < 0.05$.

1.28 mg/ml). Likewise, samples were prepared at various concentrations (0.62, 1.24, 2.48, 4.96 mg/ml).

At the concentration of 4.96 mg/ml, SA of turmeric leaf, pandan leaf and torch ginger flower in their fresh forms were 31.29%, 17.64% and 18.94% respectively while SA in their powdered forms were 98.57%, 94.00% and 97.07% respectively. Based on the plotted graph of SA, EC_{50} values were determined. However, all fresh forms had SA below 40%. Therefore, EC_{50} values (extract concentration with 50% of maximum) were unable to be detected. Contradictorily, powdered form of turmeric leaf had the highest EC_{50} value with a concentration of 0.51 mg/ml followed by powdered forms of torch ginger flower and pandan leaf with EC_{50} values of 0.46 mg/ml and 0.44 mg/ml respectively.

EC_{50} values were summarized in Table 3 and EC_{50} values of powdered forms of turmeric leaf, pandan leaf and torch ginger flower were presented in Figure 3. Since EC_{50} values could not be determined in fresh forms, comparison of scavenging activity between fresh and powdered forms was unable to be carried out. Thus, the present study found that samples in their powdered forms were better radical scavenger as compared with their respective fresh forms because the scavenging effect of fresh forms was unable to reach 50% even though concentration of samples were increased by 8 folds from the initial concentration of 0.62 mg/ml to 4.96 mg/ml.

Among all fresh and powdered forms of herbs tested, powdered forms of pandan leaf exhibited the highest scavenging activity. A study by Cousins *et al*. (2007) found that scavenging activity of *curcuma longa* following drying process was decreased as compared with its fresh forms. In contrast, this study found that scavenging activity of powdered forms of samples was better as compared with their fresh forms. This might be due to the use of different drying methods. Cousins *et al*. (2007) used oven-drying method whereas this study used freeze-drying method.

Statistical analysis showed a positive and very high ($r = 0.848$) significant correlation between TPC and SA. The finding in this study was in agreement with study by Chang *et al*. (2006), whereby they reported that the higher TPC yielded the higher SA. Similarly, a correlation between TPC and SA had been observed in different fruits and vegetables in a study done by Jimenez-Escrig *et al*. (2001).

The strong relationship between TPC and SA might be due to the combined effect of various phenolic compounds and their high hydrogen atom donating abilities. Generally, the results in this

analysis revealed that samples studied were excellent free radical inhibitors or scavenger by acting as antioxidants and hence reacting with free radicals, thereby terminating the chain reaction.

Based on the earlier discussed analysis between TPC and AA, this correlation test also suggested that phenolic compounds present in samples had stronger DPPH radical scavenging ability than ß-carotene bleaching activity. However, statistical analysis showed there was no correlation ($r = 0.200$) between AA and SA. Hence, this analysis suggested that sample with high AA does not necessary exhibit high SA and vice versa.

Conclusion

Turmeric leaf, pandan leaf and torch ginger flower exhibited phenolic compounds, antioxidant activity and scavenging activity in both fresh and powdered forms. However, antioxidant activities of powdered forms of these herbs were superior to their respective fresh forms.

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