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Effects of drying, fermented and unfermented tea of *Ocimum tenuiflorum* Linn. on the antioxidant capacity

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Abstract

Antioxidant capacity of *Ocimum tenuiflorum* L. or 'ruku' were determined in this study. Fresh leaves of *Ocimum tenuiflorum* was subjected to freeze drying, vacuum drying and processed into fermented and unfermented tea. The samples were extracted using distilled water and the total phenolics, total flavonoids, condensed tannin content, anthocyanins and total antioxidant capacity (TAC) were assessed, measured with ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) assays. The results showed that drying the fresh leaves of *Ocimum tenuiflorum* and processing them into tea leaves significantly increase ($P < 0.05$) the antioxidant capacity, total phenolic content, total flavonoid content, and condensed tannin content. However, anthocyanins content showed reduction after drying. In the present study, it can be concluded that the vacuum drying method seem to produce a product with higher quality of antioxidant properties than freeze drying. Hence, vacuum drying can be used to replace freeze drying as it is also cheaper than freeze drying.

Introduction

Some medicinal plants contain various natural antioxidants such as phenolic acids, flavonoids and tannins which are associated with higher antioxidant activity compared to that of dietary plants (Bouayed *et al*., 2007). Antioxidants are essential in the food industry due to benefits resulting from them on human health (Babovic *et al*., 2010). Aromatic herbs plants under the family Lamiaceae have high socio-economic value in medicinal preparations, flavouring, confectionery and cosmetics and perfumery (Magness *et al.*, 2006).

Plants have been thoroughly used as traditional medicines for a very long time to provide remedies and many beneficial uses to mankind and therefore, various experimental researches have been carried out to identify natural antioxidants from plants (Krishnaiah *et al.*, 2011). However, these valuable aromatic herbs are highly perishable in its fresh form and therefore have to be preserved. Water content in fresh Lamiaceae herbs is approximately 75 -80% and has to be reduced to less than 15% for preservation (Diaz-Maroto *et al*., 2002). Drying of herbs prevents microbial growth and hinders biochemical changes. At the same time, drying could result changes that might improve the herb quality such as appearance and aroma due to the loss of volatile compounds or the formation of new volatile compounds through esterification or oxidation reactions (Hossain *et al*., 2010).

Ocimum tenuiflorum Linn. is commonly known as ruku in Malaysia usually cultivated as a garden ornamental plant because of its small purplish and some yellowish flower. In Malaysia, the young leaves of *Ocimum tenuiflorum* are used to make Nasi Ulam. *Ocimum tenuiflorum* is ranked among few wonder herbs for having enormous medicinal potentialities (Kothari *et al.*, 2004). The leaves contain as essential oil, in which various compounds of medicinal value are present (Rai *et al*., 2004). Due to its manifold curative uses, the plant is considered as highly sacred in India (Kothari *et al*., 2004).

Both natural and synthetic antioxidants are used widely in the food industries. Four commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) (Jamshidin *et al*., 2012). BHA and BHT may harm human health, thus, a concentration limit of the use of these compounds is established according to regulations. Food-grade BHA, 2(3)-tert-butyl-4-hydroxyanisole is constituted of more than 85% 3-tert-butyl-4-hydroxyanisole (3-BHA) and 15% or less 2-tert-butyl-4-hydroxyanisole (2-BHA). However, food-grade BHT, 3, 5-di-tert-butyl-4-hydroxytoluene is no lesser than 99% (w/w) pure (Williams *et al*., 1999).

As synthetic antioxidants has been investigated and proven to result in toxicity, variety of researches

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have been conducted to look for more natural and alternative sources of antioxidants rather than to rely on those synthetic ones (Tachakittirungrod *et al*., 2007). The present study was conducted to determine the effect of different drying method on antioxidant capacity and the amount of total phenolic, total flavonoid, total condensed tannins and total anthocyanins in the leaves extract of *Ocimum tenuiflorum.*

Materials and Methods

Plant materials

Fresh samples of *Ocimum tenuiflorum* with no physical damage were collected from Parit Abas, Kuala Kurau, Perak. The sample was identified by Mr. Kamarudin Saleh from Herbarium Unit of Forest Research Institute Malaysia (FRIM) in Kepong, Selangor.

Preparation of samples

Leaves of the freshly obtained samples were separated from the plant and washed under running tap water followed by drying at room temperature. The first batch was used as fresh samples for solvent extraction. Another batch was dried using vacuum oven (Binder, Fisher Scientific, USA) at temperature of 30°C for 2 to 3 days. The third batch was dried using freeze drier (LD53, Kingston, New York) at temperature of -50°C for 2 to 3 days while the fourth batch of samples was dried into unfermented tea. Finally, the fifth was dried into fermented tea. The initial weight of all batches of samples was about 1 kg of leaves. The samples were then blended in a normal grinder (Panasonic MX-7995) until fine and sieved. The samples in powder form were sealed in plastic bag, kept in a dark, air-tight container and stored in a freezer at -20°C.

Preparation of unfermented Ocimum tenuiflorum *tea*

The unfermented tea preparation was adapted from *Camellia sinensis* green tea preparation as described by Rasmussen and Rhinehart (1999). The freshly plucked leaves were pan fired to halt active enzymes. They were then generally rolled (crushed) into small pieces. The leaves were allowed to dry in the pan until it reached its final stage of less than 4% of moisture.

Preparation of fermented Ocimum tenuiflorum *tea*

The fermented tea preparation was adapted from *Camellia theifera* black tea preparation described by Adisewojo (1982), consisting four steps which were withering, rolling, fermentation and drying.

Sample extraction

Extraction of sample was carried out with modification according to the method by Fu *et al.* (2011). Sample of 1 g was weighted into a conical flask wrapped with aluminium foil and 100 ml of distilled water was added into the conical flask. The mixture was shaken at 160 rpm and 27°C in an orbital shaker (Lab Companion, Model SI600R) for 24 hours. The mixture was then centrifuged (Model-4000, Kubota Corporation) at 2500 rpm for 30 minutes to obtain a clear extract.

DPPH free radical-scavenging assay

DPPH scavenging activity was done according to the method of Tabart *et al.* (2007) with slight modifications. The DPPH methanolic solution was prepared fresh by mixing DPPH with methanol at a concentration of 100 µmole/L. An aliquot (1 mL) of sample extract was mixed with 6 mL of DPPH solution. The mixture was vortexed and the mixture was kept in the dark for 30 minutes. Absorbance was read at 517 nm with UV-Vis spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer Model UV-160A) against blank of methanol. The results obtained were calculated and expressed in the term of % DPPH inhibition by using the equation 1 (Eq.1) below:

$$\%\text{inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100\% \quad \text{(Eq.1)}$$

Ferric reducing antioxidant power assay

A modified method of FRAP assay was carried out based on the method proposed by Alothman *et al.* (2009a). FRAP reagent was prepared fresh by mixing 300 mM, pH 3.6 sodium acetate buffer with 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in a volume ratio of 10:1:1. An aliquot amount of 200 µL properly diluted sample extract was mixed with 3 mL of FRAP reagent. The mixture was then incubated in water bath at 37°C for 30 minutes. Absorbencies of the samples were determined against blank at 593 nm. A standard curve was prepared by using ferrous sulphate $FeSO_4.7H_2O$ (0, 200, 400, 600, 800 µM).

Determination of total phenolic content

Total phenolic content was determined by Folin Ciocalteu (FC) assay with slight modifications as described by Alothman *et al.* (2009b). Approximately 2 mL of 10 times pre-diluted FC reagent was mixed with 400 µL properly diluted sample extract and the mixture was allowed to stand for 5 minutes at room temperature. Then, 1.6 mL of (7.5% w/v) sodium carbonate solution was added. The absorbance was read at 765 nm. A standard curve was prepared by

using gallic acid solution (0, 20, 40, 60, 80, 100 mg/L).

Determination of total flavonoid content

Colorimetric assay descibed by Alothman *et al*., (2009b) was referred to determine the total flavonoid content of the samples. Approximately 1 mL of properly diluted sample extracts was mixed with 4 mL of distilled water and at zero time, 300 µL of (5% w/v) sodium nitrite, $NaNO_2$ was added. After 5 minutes, 300 µL of 10% $AlCl_3$ was added. Subsequently, after 6 minutes, 2 mL of 1M of NaOH solution was added and the volume of the mixture was made up to 10 mL immediately by adding 2.4 mL of distilled water and the absorbance was read at 510 nm. A standard curve was prepared by using catechin solution (0, 20, 40, 60, 80, 100, 120 mg/L).

Determination of anthocyanins

The spectrophotometric pH differential method as described by Lee *et al.* (2005) was used to determine the total anthocyanins content in the extracts of the inflorescence. Extract of 0.5 ml was mixed thoroughly with 3.5 ml of 0.025 M potassium chloride buffer (pH 1). The mixture was allowed to stand for 15 minutes and the absorbance was measured at 510 nm and 700 nm against a blank of distilled water. Following the same procedure, the extract was then added to 0.4 M sodium acetate buffer (pH 4.5) and the absorbance was measured at 510 nm and 700 nm after 15 minutes. The total anthocyanin content was calculated using the following equation 2 (Eq.2):

$$\text{Total anthocyanin content (mg/L)} = (A \times MW \times DF \times 1000)/ (\varepsilon \times 1) \quad \text{(Eq.2)}$$

Where A is the absorbance of the extract calculated by equation 3 (Eq.3):

$$A = (A_{510}-A_{700})_{\text{pH 1.0}} - (A_{510}-A_{700})_{\text{pH 4.5}} \quad \text{(Eq.3)}$$

MW is the molecular weight for cyanidin-3-glucoside = 449.2; DF is the dilution factor of the samples and ε is the molar absorptivity of cyanidin-3-glucoside = 26900.

Determination of condensed tannins

Determination of condensed tannins was determined using vanillin-HCl method described by Broadhurst and Jones (1978). Briefly, 0.5 mL of the extract was added to 3 mL vanillin reagent (4 % w/v vanillin in methanol) and mixed thoroughly. Concentrated HCl (1.5 ml) was added to the mixture and vortexed. The mixture was kept in the dark for 15 minutes at room temperature and the absorbance was measured at 500 nm. A standard curve was prepared by using catechin solution (0, 20, 40, 60, 80, 100, 120 mg/L).

Statistical analysis

All of the results obtained were as means ± SD. Analysis of variance (ANOVA) was used to determine the significant differences for multiple comparisons which was completed using Duncan test at $\alpha = 0.05$. All of these were carried out using SPSS statistical package (ver.16.0).

Results and Discussion

The yield of the samples

There were no significant difference ($P > 0.05$) in the results obtained (Table 1) between the percentage yield of freeze dried and vacuum dried samples showing that the efficiency of the drying methods were relatively similar. For the tea samples, fermented tea gives significantly higher ($P < 0.05$) yield than unfermented tea. The yield of the samples increase in the following order: unfermented tea < freeze dried samples and vacuum dried samples < fermented tea. The reduced yield in freeze dried, vacuum dried and dried tea leaves samples was attributed to the loss of moisture after drying. Among the samples, unfermented tea has the lowest yield indicating that more moisture has been removed from the plant. Whereas, freeze dried, vacuum dried and fermented tea samples retained more moisture upon drying.

Table 1. Effect of different drying methods and tea processing methods on the yield of the sample (n = 3 ± S.D)

Sample of *O. tenuiflorum L*	Yield (%)
Freeze Dried	18.15^{b}±0.33
Vacuum Dried	18.08^{b}±0.15
Fermented Tea	23.45^{c}±0.59
Unfermented Tea	11.47^{a}±1.32

*Letters followed the same letter are not statistically significant from each other at $P < 0.05$; means ± standard deviation.

Effect of drying method on antioxidant capacity

This assay measures the reducing potential of antioxidants toward DPPH• radical (Price *et al*., 2001) based on the measurement of the loss of DPPH purple color at 515 nm after reaction with reaction mixture to pale yellow (Wiyekoon *et al*., 2011). Compounds like carotenoids may interfere with the analysis of result (Nomura *et al*., 1997). On the other hand, FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} to form a deep blue Fe^{2+}-TPTZ complex at 593 nm in the presence of TPTZ. High FRAP values may be associated partially to phenolic and flavonoid compounds (Riaz *et al*., 2011). FRAP is unable to detect compounds that exercise their effect by radical quenching (H transfer), particularly thiols and proteins (Ou *et al*., 2002).

According to Guo *et al.* (2003), plants with higher antioxidant capacity generally have higher amount of antioxidants. Percentage inhibition of DPPH and FRAP values showed relatively wide variation among the samples. Percentage inhibition of DPPH ranged from 24.2 ± 1.2% in vacuum dried samples to 75.0 ± 0.5% in freeze dried samples. The range of FRAP values were 33.2 ± 0.53 µM Fe(II)/g extract in fresh samples to 2912.8 ± 7.81 µM Fe(II)/g sample in unfermented tea samples.

There was a significant difference in antioxidant capacity between the different drying methods and also between the fermented and unfermented teas at $P < 0.05$. Freeze dried samples had the highest percentage inhibition of DPPH followed by fresh samples and vacuum dried samples. The results show that vacuum dried samples had significantly lowest ($P < 0.05$) percentage inhibition of DPPH while freeze dried highest ($P < 0.05$). For tea samples, unfermented tea had a higher percentage inhibition of DPPH than fermented tea. The results from the antioxidant assay showed that the extract of the plant can scavenge the radical to a certain extent.

Vacuum dried samples had the highest FRAP values followed by freeze dried samples and with fresh samples having the lowest. It was generally found that antioxidant activity was related to the total phenolics and total flavonoids content of the extracts (Table 2). For tea samples unfermented tea had a higher FRAP compared to fermented tea. FRAP is the only method that determines the antioxidant content in samples directly (Bernaert *et al*., 2010). The high FRAP value in vacuum dried samples could be due to the formation of novel compounds possessing antioxidant activity such as the Maillard reaction products (Nicoli *et al.*, 1999). Unfermented tea had the highest FRAP value which translates to the highest antioxidant activity. Both freeze dried and vacuum dried samples gave higher antioxidant capacity compared to fresh samples. The use of a vacuum may allow water to evaporate at lower temperatures and the absence of oxygen in the environment helps to reduce potential oxidation resulting in the preservation of antioxidant components (Leusink *et al*., 2010).

In the present study, results of the DPPH and FRAP assay exhibited different trends. According to Tuyen (2010), studies have proved that the results for the total antioxidant activity of dried samples is influenced by not only the drying methods utilized, but also the different antioxidant assays. Chang *et al.* (2006) demonstrated that two varieties of freeze dried tomatoes shown higher reducing power values than the hot air dried tomatoes in the reducing power assay. In contrary, hot air dried tomatoes gave the highest ferrous ion chelating ability values and also DPPH radical scavenging activity than the freeze dried and fresh tomatoes. The variation in relative antioxidant capacity between different fractions specific with DPPH and FRAP assay testing methods, respectively, can be explained by the differences in what each assay measures in evaluating antioxidant activity. For example, the DPPH assay measures the ability of compounds to act as free radical scavengers or hydrogen donors in assessing the antioxidant activity of plants (Yang *et al*., 2007). The measurement is an indirect method (Bernaert *et al*., 2012) because it merely measures the reducing ability of antioxidants toward DPPH radical (Prior *et al*., 2005).

Table 2. Effect of different drying methods and tea processing methods on the total antioxidant capacity and total phenolics, flavonoids, condensed tannins and anthocyanin content (n = 3 ± S.D)

Sample	Fresh	Freeze	Vacuum	Fermented Tea	Unfermented Tea
DPPH (% inhibition)	38.09^{b}±0.24^{b}	74.97^{c}±0.47	24.16^{a}±1.15^{a}	47.67^{c}±0.16	72.20^{d}±0.32
FRAP (µM Fe(II)/g)[A]	33.18^{a}±0.53^{a}	98.41^{b}±1.20	601.91^{d}±5.36	371.24^{c}±3.62	2912.8^{e}±7.81
Total Phenolic Content (mg GAE/100 g)[B]	273.40^{a}±2.01	1142^{b}±0.06	5957.1^{e}±5.85	3216.7^{c}±11.02	4416.1^{d}±15.76
Total Flavonoid Content (mg CE /100 g)[C]	109.02^{a}±3.12	378.81^{b}±9.17	3918^{d}±12.51	2411.1^{c}±2.46	4429.2^{e}±16.65
Condensed Tannin Content (mg CE /100 g)[C]	51.73^{a}±5.98	140.15^{c}±5.44	117.14^{b}±3.45	108.8^{b}±7.76	344.37^{d}±7.20
Total Anthocyanins (mg c-3-gE/100 g)[D]	16.29^{b}±2.39	n.d	0.44^{a}±0.10	n.d	n.d

[a]Letters followed the same letter are not statistically significant from each other at $P < 0.05$; n.d not detected; means ± standard deviation; not detected. [A] Iron(II) equivalents; [B] Gallic acid equivalents; [C] Catechin equivalents; [D] cyanidin-3-glucoside equivalents.

Efficiency of the drying methods was relatively similar with the resulting percentage yield of freeze dried and vacuum dried samples have no significance difference $p > 0.05$. Hence, the residual moisture has no influence on the antioxidant capacity of the samples. Similar findings have been reported by Hossain *et al.* (2010). As for tea samples, antioxidants in fermented tea leaves were nearly exhausted after processing whereas in unfermented tea, most of its antioxidants were retained in the leaves after processing. This suggested that fermented tea has a lower level of antioxidant than unfermented tea (Hlahla *et al*., 2010).

Total phenolic content

Generally, there is always dispute over what is being detected in total antioxidant capacity assays, whether it is only phenols or phenols plus reducing agents and possibly metal chelators being detected (Prior *et al.*, 2005). Total phenolics content assay has been commonly used to measure total phenolic content in natural materials, but the basic mechanism is a reduction or oxidation based reaction and can be as an alternative to calculate the protective effects of antioxidants. This method is uncomplicated, sensitive and accurate (Prior *et al*., 2005).

The degree to which a particular phenolic compounds contribute to the total antioxidant capacity

of plant depend on not only the relative concentration of individual antioxidant compounds, but also the possible synergistic interactions that occur between the various plant constituent (Riaz *et al*., 2011). Generally, antioxidant mechanism of polyphenols is based on their hydrogen donating and metal ion chelating abilities (Lee *et al*., 2005). Skerget *et al.* (2005) stated that the antioxidant activity of most plants is mainly due to the presence of phenolic compounds.

According to the results in Table 2, it was evident that vacuum dried samples had the highest total phenolic content (5957.1 mg GAE/100 g), followed by freeze dried samples (1142 mg GAE/100 g) and fresh samples (273.4 mg GAE/100 g). For tea samples, unfermented tea had a higher total phenolic content (4416.1 ± 15.76 mg GAE/100 g) than fermented tea (3216.7 ± 11.02 mg GAE/100 g). There was a significant difference at $p < 0.05$ in total phenolic content between the different drying samples and also between the fermented and unfermented teas. It was observed that drying and processing leaves into tea resulted in a considerable increase of total phenolic content.

Phenolic compounds could be released from these collapsed cell walls into the solvent. Drying treatments helps to release large amount of bound phytochemicals into the medium, namely phenolic compounds from matrix, thus making them more accessible by extraction solvent as well (Dorta *et al*., 2012). One reason causing fresh samples to have the lowest total phenolic content is that the antioxidants might have been lost through degradation by enzymes that are still active (Hossain *et al*., 2010). Suhaj (2006) proposed the use of dry, frozen or lyophilized plant materials as some antioxidants in the fresh undried samples could be unstable or degraded by enzymatic action. In dried samples, due to the decreased water content, enzymes were inactivated and unable to degrade the antioxidants. Therefore, more antioxidants are retained, thus maintaining a high antioxidant capacity and total phenols in the extracts (Hossain *et al*., 2010). Dorta *et al.* (2012) concluded that plant material stabilized by drying did not only conserve the plant but also enhance the antioxidant properties and the content of bioactive components.

Vacuum dried samples showed the highest total phenol content and antioxidant capacity of all the samples. For vacuum drying, the fresh herbs were kept at 30°C and it took three days to get the samples to dry completely. The plants lost moisture slowly during this period and might have detected the moisture loss as stress (Hossain *et al*., 2010). In response to this stress, more phenolic compounds were produced as a defence mechanism (Rhodes *et al*., 1985). Comparing both drying method, freeze dried sample seems to have lower total phenolic content than vacuum dried sample. Freeze drying fails to preserve fully and may diminish some important classes of plant bioactive compounds, namely phenolics and other volatiles (Abascal *et al*., 2005).

For the tea samples, unfermented tea showed higher total phenolic content compared to fermented tea probably because in unfermented tea, polyphenol oxidase was inactivated by drying and thus oxidation of polyphenols were inhibited. Hence, more phenolics were retained in the unfermented tea. The fact that fermented tea has lower total phenolic content may be due to the formation of colour and flavour compounds during fermentation which reduced the polyphenol concentration (Mahanta *et al*., 2010).

Total flavonoid content

Flavonoids compounds present in plants are believed to be good natural antioxidants (Sun *et al*., 2011). These bioactive defense substances are also produced in response to environmental stress such as temperature fluctuations, UV radiation and air pollution (Maimoona *et al*., 2011).

The flavonoid content ranged from 109.02 mg CE/100 g in fresh sample to 4429.2 mg CE /100 g in unfermented tea samples (Table 2). The flavonoid content was significantly higher ($P < 0.05$) in both vacuum oven and freeze dried samples compared to fresh samples. The results showed that drying the sample at a temperature of 30°C in vacuum oven guarantees higher flavonoid content than freeze drying. Tea samples also had a significantly higher ($P < 0.05$) flavonoid content compared to fresh leaves with unfermented tea having significantly higher ($P < 0.05$) flavonoid content than fermented tea.

The significant increase ($P < 0.05$) in flavonoid content in all the dried samples and also teas samples may be attributed to the formation of more flavonoid compounds in response to stress due to moisture loss. Dixon and Paiva (1995) reported that biotic and abiotic stress factors such as wounding, low temperature, pathogen attacks may triggered the synthesis of phenylpropanoid compounds such as flavonoids, isoflavonoids, psoralens, coumarins, phenolic acids, lignin and suberin.

Anthocyanins content

Anthocyanins fall under the widespread class of phenolic compounds collectively known as flavonoids (Bakowska-Barczak, 2005). There is an intensified interest in the anthocyanins level in foods

and nutraceuticals because of the possible health benefits. Anthocyanins produced the red, purple and blue colours in fruits, vegetables and grains. There are six common anthocyanidins, namely, pelargonidin, cyaniding, peonidin, delphinidin, petunidin and malvidin (Lee *et al.*, 2005). Besides contributing to the colour attributes, anthocyanins are important due to their possible health benefits (Bąkowska-Barczak, 2005).

The results in Table 2 showed that anthocyanins content in the fresh samples present at 16.29 ± 2.39 mg c-3-gE/100 g. Since, the level of anthocyanins in the fresh sample was low, the participation of anthocyanins in the total antioxidant capacity of the plant seems to be of lesser importance. According to the results, both freeze drying and vacuum drying had led to deterioration of anthocyanins content in the fresh samples. The anthocyanins content was 0.44 ± 0.1 mg c-3-gE/100 g in vacuum dried sample and was not detected in freeze dried sample. The slight anthocyanins content present in vacuum dried sample compared to freeze dried sample indicates that anthocyanins may be more stable at higher temperature during drying and favours moderate drying condition in terms of temperature (Komes *et al*., 2011). For tea samples, anthocyanin was not detected in both the fermented and unfermented teas.

Condensed tannin content

Tannins obtain their name from the fact that they have the capability to bind and crosslink protein and their application in the tanning process animal skin (Häring *et al*., 2007). Several crucial properties of condensed tannins include their ability to form complex with metal ions, antioxidant and radical scavenging activities and complexation with macromolecule, such as proteins, carbohydrates, polysaccharides and cell membranes (Haslam, 1996; Su *et al*., 1988).

The vanillin assay that had been used in this study is based on the reaction between an aromatic aldehyde and catechin, the standard monomer of condensed tannins also reacts in the assay and results in the formation of a red coloured adduct (Hagerman, 2002). Methanol is commonly used as solvent in the vanillin assay as in methanol, vanillin reaction is more receptive toward polymeric condensed tannins compared to monomeric flavanols (Price *et al.*, 2001). However, an overestimation of condensed tannin content will still occur in crude plant extracts that is rich in monomeric compounds (Chavan *et al*., 2001). Also, methanol lessens the colour yield of the reaction and cause less interference. Under normal conditions, proanthocyanindins and catechin both react with vanillin in the vanillin assay (Hagerman, 2002).

The total condensed tannin content was 51.73 mg CE/100 g in the fresh sample and was significantly higher ($P < 0.05$) in vacuum and freeze dried samples and also the teas (Table 2). Freeze and vacuum dried samples contained twice the total condensed tannin than that of fresh sample. Comparing the dried plant samples, freeze dried sample had a slightly higher total condensed tannin content than vacuum dried sample. According to Abascal *et al*. (2005), freeze drying is a good method to preserve large molecular weight condensed tannins. For tea samples, unfermented tea has relatively higher total condensed tannin content than fermented tea.

In conclusion, the effect of different drying methods on the total antioxidant capacity, phenolics, flavonoids, anthocyanins, and condensed tannin contents may be fundamental for future investigation. Further studies to determine the sensory quality parameters of tea, namely taste and aroma may be carried out since they are the most dominant parameters for the determination of the tea quality. In the present study, it can be concluded that the vacuum drying method produce a product with higher quality of antioxidant properties than freeze drying. Hence, vacuum drying can be used to replace freeze drying as it is also cheaper than freeze drying. By analyzing these herbs, more opportunities are created to boost the use of Malaysian local herbs in food preparations not only in medicine field but also in household food preparation. The findings in this experiment can also be used as a reference for future research on the dietary intake level of these herbs.

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References

Abascal, K., Ganora L. and Yarnell, E. 2005. The effect of freeze-drying and its implications for botanical medicine: A Review. Phytotherapy Research 19: 655–660.

Adisewojo, R.S. 1982. Bercucuk tanam the (*Camellia theifera*), Penerbitan Summur Bandung.

Alothman, M., Bhat, R. and Karim, A. A. 2009a. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry 115: 785-788.

Alothman, M., Bhat, R. and Karim, A. A. 2009b. UV

radiation-induced changes of antioxidant capacity of fresh-cut tropical fruits. Innovative Food Science and Emerging Technologies 10: 512–516.

Babovic, N., Djilas, S., Jadranin, M., Vajs, V., Ivanovic, J., Petrovic. and Zizovic, I. 2010. Supercritical carbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capacity. Innovative Food Science and Emerging Technologies 11: 98–107.

Bakowska-Barczak, A. 2005. Acylated anthocyanins as stable, natural food colorants- A review. Polish Journal of Food and Nutrition Sciences 14/55 (2): 107–116.

Bernaert, N., De Paepe, D., Bouten, C., Declercq, H., Stewart, D., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. 2012. Antioxidant capacity, total phenolic and ascorbate content as a function of the genetic diversity of leek (*Allium ampeloprasum* var. porrum), Food Chemistry 134 (2): 669-77.

Bouayed, J., Piri, K., Rammal, H., Dicko, A., Desor, F., Younos, C. and Soulimani, R. 2007.Comparative evaluation of the antioxidant potential of some Iranian medicinal plants, Food Chemistry 104: 364–368.

Broadhurst R.B. and Jones W.T. 1978. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture 29: 788-794.

Chang, C., Lin, H., Chang, C. and Liu, Y. 2006. Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. Journal of Food Engineering 77: 478–485.

Chavan, U.D., Shahidi, F. and Naczk, M. 2001.Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. Food Chemistry 75: 509–512.

Diaz-Maroto, M. C., Perez Coello, M. S. and Cabezudo, M. D. 2002. Effect of different drying methods on the volatile components of parsley (*Petroselinum crispum* L.). European Food Research Technology 215: 227-230.

Dixon, R. A. and Paiva, N. L.1995. Stress-lnduced Phenylpropanoid Metabolism. The Plant Cell 7: 1085-1097.

Dorta, E., Lobo, M. G. and González, G. 2012. Using drying treatments to stabilise mango peel and seed: Effect on antioxidant activity. LWT - Food Science and Technology 45: 261-268.

Fu, L., Xu, B., Xu, X., Gan, R., Zhang, Y., Xia, E. and Li, H. 2011. Antioxidant capacities and total phenolic contents of 62 fruits. Food Chemistry 129: 345–350.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J. and Jiang, Y. 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research 23: 1719–1726.

Hagerman, A.E. 2002. Tannin Handbook. Miami University, Oxford OH, 45056 *www.users.muohio.edu/hagermae/*.

Häring, D. A. 2007. Determinants of tannin concentrations in forage plants. Swiss Federal Institute of Technology, Zurich: ETH, Doctoral and Habilitation Theses.

Haslam, E. 1996. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. Journal of Natural Products 59 (2): 205-215.

Hlahla, L. N. 2010. Effect of fermentation temperature and duration on chemical composition of bush tea (*Athrixia phylicoides* DC.), South Africa: University of Limpopo, Msc Thesis.

Hossain, M. B., Barry-Ryan, C., Martin-Diana, A. B. and Brunton, N. P. 2010. Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. Food Chemistry 123: 85–91.

Jamshidian, M., Tehrany, E. A., Cleymand, F., Leconte, S., Falher, T. and Desobry, S. 2012. Effects of synthetic phenolic antioxidants on physical, structural, mechanical and barrier properties of poly lactic acid film. Carbohydrate Polymers 87: 1763– 1773.

Komes, D., Belščak-Cvitanović, A., Horžić, D., Marković, K. and Ganić, K. K. 2011. Characterisation of pigments and antioxidant properties of three medicinal plants dried under different drying conditions. Proceedings of the 11th International Congress on Engineering and Food.

Kothari, S.K., Bhattacharya, A.K. and Ramesh, S. 2004. Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L.f. (syn. *O. sanctum* L.) grown in south India as influenced by method of harvest. Journal of Chromatography A 1054: 67-72.

Krishnaiah, D., Sarbatly, R. and Nithyanandam, R. 2011. A review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing 89: 217–233.

Lee, J., Durst, R. W. and Wrolstad, R. E. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative Study. Journal of AOAC International 88 (5): 1269-1278.

Lee, J., Koo, N. and Min, D.B. 2004. Reactive oxygen species, aging and antioxidative nutraceuticals. Comprehensive Reviews in Food Science and Food Safety 3: 21–33.

Leusink, G. J., Kittis, D. D., Yaghmaee, P. and Durance, T. 2010.Retention of antioxidant capacity of vacuum microwave dried cranberry. Journal of Food Science 75(3): C311-C316.

Magness, J.R., Markle, G.M. and Compton, C.C. 2006. Food and feed crops of the United States. Interregional Research Project IR-4, IR Bul. 1, (Bulletin 828 of the New Jersey Agricultural Experimental Station). Revised Edition. American Botanical Council.

Mahanta, P.K. and Hazarika, M. 1985. Chlorophyll and degradation products in orthodox and CTC black teas and their influence on shade of colour and sensory quality in relation to thearubigins. Journal of the Science of Food and Agriculture 36: 1133-1139.

Maimoona, A., Naeem, I., Saddiqe, Z., Ali, N., Ahmed, G. and Shah, I. 2011. Analysis of total flavonoids and phenolics in different fractions of bark and needle extracts of *Pinus roxburghii* and *Pinus wallichiana*. Journal of Medicinal Plants Research 5(13): 2724-2728.

Nicoli, M.C., Anese, M. and Parpine, M. 1999. Influence of processing on the antioxidant properties of fruit and

vegetables. Trends in Food Science and Technology 10: 94-100.

Nomura, T., Kikuchi, M., Kubodera, A. and Kawakami, Y. 1997. Proton-donative antioxidant activity of fueoxanthin with 1,1-diphenyl-2-Picrylhydrazyl (DPPH). Biochemistry and Molecular Biology International 42(2): 361-370.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A. and Deemer, E. K. 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) Assays: A Comparative Study. Journal of Agricultural and Food Chemistry 50: 3122-3128.

Price, M. L., Van Scoyoc, S. and Butler, L. G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry 26: 1214–1217.

Prior, R. L., Wu, X. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53: 4290-4302.

Rai, V. Vajpayee, P., Singh, S.N. and Mehrotra, S. 2004. Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. Plant Science 167: 1159-1169.

Rasmussen, W. and Rhinehart, R.1999. Tea basics: A quick and easy guide, New York : John Sons, Inc.

Rhodes, M. J. C. 1985. The physiological significance of plant phenolic compounds. The biochemistry of plant phenolics. In Annual proceedings of the phytochemical society of Europe, p. 99–117. Oxford, UK: Oxford University Press.

Riaz, T., Abbasi, M. A., Aziz-ur-Rehman., Shahzadi, T., Qureshi, M. Z. and Khan, K. M. 2011. Antioxidant activity and radical scavenging effects of various fractions from *Curcuma zedoaria*. Asian Journal of Pharmaceutical and Biological Research 1(4): 525-533.

Škerget, M., Kotnik, P., Hadolin, M., Hraš., Simonič, M. and Knez, Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry 89: 191–198.

Su, J. D., Osawa, T., Kawakishi, S. and Namiki, M. 1988.Tannin antioxidants from *Osbeckia chinensis.* Phytochemistry 27 (5): 1315-1319.

Suhaj, M. 2006. Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis 19: 531–537.

Sun,L., Zhang, J., Lu, X., Zhang, L. and Zhang, Y. 2011. Evaluation to the antioxidant activity of total flavonoids extract from persimmon (*Diospyros kaki* L.) leaves. Food and Chemical Toxicology 49: 2689–2696.

Tabart, J., Kevers, C., Sipel, A., Pincemail, J., Defraigne, J. and Dommes, J. 2007. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chemistry 105: 1268–1275.

Tachakittirungrod,S.,Okonogi,S. andChowwanapoonpohn, S. 2007. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chemistry 103: 381–388.

Tuyen, C. K. 2010. Effects of different drying processes on the physicochemical and antioxidant properties of Gac fruit powder. Australia: University of Newcastle, M. Phil. Thesis

Wijekoon, M. M. J. O., Bhat, R. and Karim, A. A. 2011.Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etlingera elatior* Jack.) inflorescence. Journal of Food Composition and Analysis 24: 615–619.

Williams, G. M., Iatropoulos, M. J. and Whysner, J. 1999. Safety Assessment of Butylated Hydroxyanisole and Butylated Hydroxytoluene as Antioxidant Food Additives. Food and Chemical Toxicology 37: 1027-1038.

Yang, Z., Xu, Y., Jie, G., He, P. and Tu, Y. 2007.Study on the antioxidant activity of tea flowers (*Camellia sinensis*). Asia Pacific Journal of Clinical Nutrition 16: 148-152.