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Effects of Different Drying Methods on the Antioxidant Activities of Leaves and Berries of *Cayratia trifolia*

(Kesan Kaedah Pengeringan yang Berbeza Terhadap Aktiviti Antioksida Daun dan Beri *Cayratia trifolia*

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ABSTRACT

This study aimed to assess the effects of fresh, thermal drying method (vacuum oven drying), and nonthermal drying method (freeze drying) on the antioxidant activities of leaves and berries of Cayratia trifolia *using ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) assays. The total phenolic content (TPC) and flavonoid content (TFC) of the leaves and berries of* C. trifolia *were also measured. Based on the results obtained, the TPC, TFC, and antioxidant activities of the leaves and berries were arranged in the following order: freeze-dried sample with methanol extraction > vacuum-dried sample with methanol extraction > freeze-dried sample with water extraction > vacuum-dried sample with water extraction > fresh sample with methanol extraction > fresh sample with water extraction. The results showed a significant difference (*$p<0.05$*) between the fresh and dried samples. In conclusion, freeze drying was found to be a good method for maintaining TPC, TFC, and antioxidant activities by FRAP and DPPH methods in the leaves and berries of* C. trifolia.

Keywords: Cayratia trifolia; *drying method; total antioxidant activity; total flavonoid; total phenolic*

ABSTRAK

Kajian ini bertujuan menilai kesan kaedah pengeringan yang berbeza terhadap sampel segar, kaedah pengeringan terma (pengeringan ketuhar vakum) dan kaedah pengeringan tak terma (pengeringan beku) ke atas aktiviti antioksida daun dan beri Cayratia trifolia *yang diukur melalui asai kuasa antioksidan penurunan ferik (FRAP) dan asai 1,1-difenil-1-pikrilhidrazil (DPPH). Jumlah kandungan fenolik (TPC) dan flavonoid (TFC) juga diukur. Berdasarkan keputusan yang diperolehi, jumlah kandungan fenolik, flavonoid dan jumlah aktiviti antioksidan dalam daun dan beri boleh disusun seperti berikut: Sampel yang dikering secara pembekuan dengan pengekstrakan metanol > sampel yang dikering secara oven vakum dengan pengekstrakan metanol > sampel yang dikering secara pembekuan dengan pengekstrakan air > sampel yang dikering secara oven vakum dengan pengekstrakan air > sampel yang segar dengan pengekstrakan metanol > sampel yang segar dengan pengekstrakan air. Keputusan menunjukkan perbezaan yang signifikan (*$p<0.05$*) antara sampel segar dan kering. Kesimpulannya, pengeringan pembekuan dikenal pasti sebagai kaedah yang baik untuk mengekalkankan jumlah kandungan fenolik, flavonoid dan aktiviti antioksidan melalui kaedah FRAP dan DPPH dalam daun dan beri* C. trifolia.

Kata kunci: Cayratia trifolia*; jumlah aktiviti antioksida; jumlah fenolik; jumlah flavonoid; kaedah pengeringan*

INTRODUCTION

To date, synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene are widely used in the food industry because of their abilities to prevent food deterioration and extend the shelf life of foods (Hue et al. 2012). However, using synthetic antioxidants has negative effects, such as increase risk of cancer occurrence and liver damage in human (Bjelakovic et al. 2007). Therefore, searching for alternative sources of natural antioxidant is becoming increasingly important.

Cayratia trifolia is commonly known as Lakom in Malay, Fox grape in English and kalit-kalit in Filipino. This liana plant is found at low altitudes and native to Asia and Australia (Kumar et al. 2011). It possesses various physiological effects, including wound healing and antibacterial and diuretic actions (Gupta & Tandon 2007; Kumar et al. 2011). *C. trifolia* is used as a medical herb, but not extensively investigated. Moreover, its antioxidant content is rarely reported. Given these reasons, continuous effort is necessary to discover more effective antioxidants from natural sources as alternatives to synthetic food additives to maintain and improve health and wellness.

Different drying and extraction methods can alter the estimation of antioxidant activity in a sample (Siddhuraju & Becker 2003; Sultana et al. 2007; Thurkmen et al. 2006). In this regard, several treatments such as freeze drying, vacuum oven drying and using a fresh sample with water and methanol extractions have been used to estimate the antioxidant content in the leaves and berries of *C. trifolia*.

Materials and Methods

Plant material and sample preparation

Leaves and berries of *C. trifolia* were freshly collected from Kuala Kurau, Perak, Malaysia at the end of December 2011 and January 2012 and identified by Mr. Adnan Jaafar. Voucher specimen (USM Herbarium 11398) was deposited in the herbarium of School of Biological Sciences, Universiti Sains Malaysia. The samples were separated into three groups, which were used as freshly blended, freeze dried and vacuum oven dried.

The samples were dried using freeze drier (LD53, Kingston, New York) at temperature of -50°C for 2 to 3 days. Another batch was dried using vacuum oven (Binder, Fisher Scientific, USA) at temperature of 30°C for 2 to 3 days.

For the freshly blended sample, the blended leaves and berries were maintained in a plastic container and refrigerated at 4°C for not more than 1 week. For the freeze- and oven vacuum-dried samples, the leaves and berries were ground to a fine powder with an electric grinder and maintained in dark air-tight plastic containers. The samples were stored in a freezer at -20°C before further analysis was carried out.

Sample extraction

Sample extraction was performed following the method of Ikram et al. (2009) with modification. The extract was obtained by mixing 1 g of sample with 100 mL of 80% methanol (v/v) in a conical flask wrapped with aluminum foil. The mixture was shaken overnight in an orbital shaker (Lab Companion, Model SI600R) at 160 rpm and 27°C. The mixture was then centrifuged (Kubota, Model 4000) at 2500 rpm for 30 min to obtain a clear solution. These steps were repeated using distilled water instead of methanol for water extraction.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging assay

DPPH assay was adapted and modified from Tabart et al. (2007). About 1 mL of sample extract (in methanol) was mixed with 6 mL of 100 μmol/L DPPH solution. Positive control was prepared by mixing 6 mL of DPPH with 1 mL of standard and blank was prepared using 6 mL of methanol. Negative control was prepared by mixing 6 mL of DPPH with 1 mL of methanol. The absorbance was measured at 517 nm using a UV-vis spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer, Model UV-160A) against blank. The results obtained were calculated and expressed in % of DPPH free radical scavenging activity using (1):

$$\%\text{ of DPPH free radical scavenging activity} = \frac{AC - AS}{AC} \times 100\%, \quad (1)$$

where AS is the absorbance of DPPH radical in the presence of sample and AC is the absorbance of DPPH radical without sample (negative control).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was conducted according to Benzie and Strain (1996) with some modifications. Approximately 200 μL of properly diluted sample extracts was allowed to react with 3 mL of FRAP reagent for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were obtained by the spectrophotometer at 593 nm. The standard curve was linear between 200 and 800 μmol/L ferrous sulphate $FeSO_4 \cdot 7H_2O$.

Determination of total phenolic content (TPC)

The TPC of the sample extracts was determined with the Folin–Ciocalteu (FC) spectrophotometric method adopted from Singleton and Rossi (1956) by using gallic acid as standard phenolic compound. About 400 μL of properly diluted extract was mixed with 2 mL of 10× diluted FC reagent. After 5 min, 1.6 mL of 7.5% sodium carbonate was added and allowed to stand for 1 h at room temperature. The absorbance of the resulting blue-colored solution was measured at 765 nm.

Determination of total flavonoid content (TFC)

The TFC was determined using a modified aluminum chloride colorimetric method described by Jia et al. (1999) using catechin as standard. Approximately 1 mL of properly diluted sample extract was mixed with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. After 5 min, 0.3 mL of 10% aluminium chloride solution was added to the mixture. Subsequently, after 6 min, 2 mL of 1 M sodium hydroxide was added and the total volume was adjusted to 10 mL with distilled water. The solution was mixed well again and the absorbance was measured against a blank at 510 nm. The standard curve was linear between 20 and 120 mg/L catechin. The results were expressed as mg catechin equivalent per gram sample.

Statistical Analysis

The results were reported as means ± SD for triplicate determinations. ANOVA and the significant differences between mean values were determined using the Duncan test at $p<0.05$. Statistical analyses were conducted with SPSS 16.0 (SPSS for Windows, 2007, SPSS Inc., Chicago, IL, USA).

Results and Discussion

DPPH assay

The DPPH assay was used to evaluate the ability of antioxidants to scavenge free radicals and considered as a standard and simple colorimetric method (Mishra et al. 2012). This method is based on the reduction of DPPH in methanol solution in the presence of hydrogen-donating antioxidant because of the formation of the non-radical form DPPH-H in the reaction (Lavanya et al. 2012).

The dark color of DPPH radical solution became lighter and in the presence of an antioxidant compound, the absorbance of the solution at 517 nm decreased as the reaction between antioxidant molecules and DPPH proceeded. The absorbance read at 517 nm was used as a measure of the inhibition effect of a particular extract for DPPH radicals (Mishra et al. 2012).

Table 1 shows the result of DPPH assay obtained in mean and standard deviation. The results from this study showed that all samples exhibited antioxidant activity in varying degrees, ranging from (41.40±0.64) to (92.44±0.20) % of DPPH inhibition. The samples, which were freeze dried and extracted using methanol as solvent, generally showed higher percentage of DPPH inhibition.

The percentage of DPPH inhibition in berries was higher than that in leaves. Freeze-dried berries with methanol extraction (92.44±0.20%) exhibited the highest percentage of DPPH inhibition, followed by vacuum oven-dried berries with methanol extraction (92.10±0.39%), freeze-dried berries with water extraction (90.04±0.25%), vacuum oven-dried berries with water extraction (89.64±0.50%) and fresh berries with methanol extraction (61.47±0.98%). Fresh berries with water extraction (42.22±0.40%) showed a minimum percentage. A significant difference at $p<0.05$ was observed between the fresh and dried berry samples.

Freeze-dried leaves with methanol extraction (90.48±0.45%) showed the highest percentage of DPPH inhibition, followed by vacuum oven-dried leaves with methanol extraction (90.05±0.33%), vacuum oven-dried leaves with water extraction (88.41±0.28%), freeze-dried leaves with water extraction (78.87±1.01%), fresh leaves with methanol extraction (62.92±0.07%) and fresh leaves with water extraction (41.40±0.64%). Siriwatanametanon et al. (2010) reported that the methanol extract of the fresh leaves of *C. trifolia* has more potent DPPH free radical scavenging activity and strongly inhibits lipid peroxidation compared with *Pouzolzia indica*. A significant difference at $p<0.05$ was found between the fresh and dried leaf samples using water extraction.

FRAP ASSAY

When the samples reacted with FRAP solution, a dark blue color of the solution appeared, which refers to the ferrous tripyridyltriazine complex (Benzie & Strain 1996). This complex was detected at 593 nm. The reaction is linearly related to the molar concentration of the antioxidants. The extracts, which exhibited high antioxidant, produced more ferrous tripridyltriazine complexes.

Table 2 shows the obtained results of FRAP assay, which were expressed in mean and standard deviation. All samples showed antioxidant activity in varying degrees, ranging from (71.7±7.6) µmol Fe(II)/g of sample extract to (3515.0±117.6) µmol Fe(II)/g of sample extract. The samples were extracted using methanol as solvent generally showed higher FRAP values. Freeze-dried leaves with methanol extraction (3515.0±117.6 µmol Fe(II)/g of sample extract) showed a maximum FRAP value, followed by vacuum oven-dried leaves with methanol extraction (1528.3±77.5 µmol Fe(II)/g of sample extract), freeze-dried leaves with water extraction (1521.7±162.7 µmol Fe(II)/g of sample extract), vacuum oven-dried leaves with water extraction (953.33±55.1 µmol Fe(II)/g of sample extract), fresh leaves with water extraction (143.3±7.6 µmol Fe(II)/g of sample extract) and fresh leaves with methanol extraction (71.7±7.6 µmol Fe(II)/g of sample extract). A significant difference at $p<0.05$ was observed between the fresh, vacuum oven- and freeze-dried samples.

Freeze-dried berries with methanol extraction showed the highest FRAP value (696.0 ± 5.3 µmol Fe(II)/g of sample extract), followed by vacuum oven-dried berries with methanol extraction (569.9±8.5 µmol Fe(II)/g of sample extract), freeze-dried berries with water extraction (554.7±14.2 µmol Fe(II)/g of sample extract), vacuum oven-dried berries with water extraction (537.0±31.4 µmol Fe(II)/g of sample extract), fresh berries with methanol extraction (57.7±12.0 µmol Fe(II)/g of sample extract) and fresh berries with water extraction (47.1±6.5 µmol Fe(II)/g of sample extract).

TPC

Freeze-dried leaves with methanol extraction had the highest phenolic content (73.3±1.6 mg GAE/g sample), followed by vacuum oven-dried leaves with methanol extraction (67.7±2.5 mg GAE/g sample), vacuum oven-dried leaves with water extraction (53.6±1.2 mg GAE/sample), freeze-dried leaves with water extraction (53.2±3.1 mg GAE/g sample) and fresh leaves with water

TABLE 1. DPPH inhibitions (%) of the samples by using different solvents extraction and drying methods

Sample	Extraction method	Fresh	Drying method	
			Vacuum-oven drying % inhibition of DPPH	Freeze drying
Leaf of *Cayratia trifolia*	Water	41.40±0.64[a]	88.41±0.28[c]	78.87±1.01[b]
	methanol	62.92±0.07[b]	90.05±0.33[d]	90.48±0.45[d]
Berry of *Cayratia trifolia*	Water	42.22±0.40[a]	89.64±0.50[c]	90.04±0.25[c]
	methanol	61.47±0.98[b]	92.10±0.39[d]	92.44±0.20[d]

[1]Values are means ± SD (n=3). For each row, values followed by different letter are significantly different at $p< 0.05$

TABLE 2. Ferric-reducing power of the samples by using different solvents extraction and drying methods

Sample	Extraction method	Fresh	Drying method	
			Vacuum-oven drying μmol Fe(II) per g	Freeze drying
Leaf of *Cayratia trifolia*	Water	143.3±7.6[a]	953.33±55.1[b]	1521.7±162.7[c]
	methanol	71.7±7.6[a]	1528.3±77.5[c]	3515.0±117.6[d]
Berry of *Cayratia trifolia*	Water	47.1±6.5[a]	537.0±31.4[b]	554.7±14.2b[c]
	methanol	57.7±12.0[a]	569.9±8.5[c]	696.0±5.3[d]

[1]Values are means ± SD (n=3). For each row, values followed by different letter are significantly different at p<0.05

TABLE 3. Total phenolic content of the samples by using different solvents extraction and drying methods

Sample	Extraction method	Fresh	Drying method	
			Vacuum-oven drying mg GAE per g	Freeze drying
Leaf of *Cayratia trifolia*	Water	8.0±0.1[a]	53.6±1.2[b]	53.2±3.1[b]
	methanol	7.7±0.3[a]	67.7±2.5[c]	73.3±1.6[d]
Berry of *Cayratia trifolia*	Water	2.9±0.1[a]	24.7±0.6[c]	30.8±0.3[d]
	methanol	4.6±0.3[b]	25.9±1.0[c]	45.1±1.5[e]

[1]Values are means ± SD (n=3). For each row, values followed by different letter are significantly different at p<0.05

extraction (8.0±0.1 mg GAE/g sample). Fresh leaves with methanol extraction (7.7±0.3 mg GAE/g sample) (Table 3) showed the lowest content. The samples with methanol extraction had higher TPC compared with water extraction. Generally, the results obtained showed that a significant difference at p<0.05 existed among fresh, vacuum oven- and freeze-dried samples.

Freeze-dried berries with methanol extraction showed the highest phenolic content (45.1±1.5 mg GAE/g sample), followed by freeze-dried berries with water extraction (30.8±0.3 mg GAE/g sample), vacuum oven-dried berries with methanol extraction (25.9±1.0 mg GAE/g sample), vacuum oven-dried berries with water extraction (24.7±0.6 mg GAE/g sample) and fresh berries with methanol extraction (4.6±0.3 mg GAE/g sample). Fresh berries with water extraction (2.9±0.1 mg GAE/g sample) showed the lowest content. The results showed that significant difference at p<0.05 existed among fresh, vacuum oven- and freeze-dried samples.

The methanol extract of the leaves of *C. trifolia* showed the highest antioxidant activity because of the high amount of phenolics, such as cyanic acid and cyaniding (Siriwatanametanon et al. 2010). Maisuthisakul et al. (2007) reported that phenolic compounds and its derivatives, such as phenolic acids and tannins, are strongly correlated with antioxidant activity.

TFC

Table 4 shows that leaf samples had higher TFC than berry samples. The main flavonoids in foods are kaempferol and quercetin (Manach et al. 2004). Similar to TPC, the TFC varied with the solvent used for extraction, with methanol extract showing higher flavonoid content in plants.

Freeze-dried leaves with methanol extraction had the highest TFC (45.0±16.5 mg CE/g sample), followed by vacuum oven-dried leaves with methanol extraction (34.1±0.8 mg CE/g sample), vacuum oven-dried leaves with water extraction (25.1±1.3 mg CE/g sample), freeze-dried leaves with water extraction (18.0±0.9 mg CE/g sample) and fresh leaves with water extraction (5.8±0.1 mg CE/g sample). Fresh leaves with methanol extraction (5.7±0.1 mg CE/g sample) showed the lowest TFC. The high content of flavonoid was strongly related to the potent of DPPH and FRAP ferric reducing value. Between the fresh, freeze- and vacuum oven-dried leaves, a significant difference at p<0.05 was found.

Freeze-dried berries with methanol extraction had the highest TFC (16.4±0.2 mg CE/g sample), followed by freeze-dried berries with water extraction (14.4±0.2 mg CE/g sample), vacuum oven-dried berries with methanol extraction (11.1±0.7 mg CE/g sample), vacuum oven-dried berries with water extraction (11.0±0.4 mg CE/g sample) and fresh berries with methanol extraction (1.8±0.3 mg CE/g sample). Fresh berries with water extraction (1.0±0.1 mg CE/g sample) had the lowest TFC. A significant difference at p<0.05 was found between the fresh, freeze- and vacuum oven-dried berries.

CONCLUSION

In general, the antioxidant activity in freeze-dried samples was higher than that in the vacuum oven-dried and fresh samples. Hossain et al. (2010) reported that relatively low antioxidant estimation in fresh samples had a very strong correlation with high moisture content, which caused dilution effect toward the total antioxidant content in fresh

TABLE 4. Total flavonoid content of the samples by using different solvents extraction and drying methods

Sample	Extraction method	Fresh	Drying method	
			Vacuum-oven drying	Freeze drying
			mg CE per g	
Leaf of *Cayratia trifolia*	Water	5.8±0.1[a]	25.1±1.3[bc]	18.0±0.9[ab]
	methanol	5.7±0.1[a]	34.1±0.8[cd]	45.0±16.5[d]
Berry of *Cayratia trifolia*	Water	1.0±0.1[a]	11.0±0.4[c]	14.4±0.2[d]
	methanol	1.8±0.3[b]	11.1±0.7[c]	16.4±0.2[e]

[1]Values are means ± SD (n=3). For each row, values followed by different letter are significantly different at p<0.05

samples. Fresh and high moisture contents of samples may also lose its antioxidant compounds through the enzymatic degradation process because the active enzymes in fresh samples are still high.

Previous investigations (Chan et al. 2009; Di Cesare et al. 2003; Hsu et al. 2003) reported that freeze-dried samples have high result in antioxidant estimation. Freeze drying has high efficiency in moisture removal and maintains bioactive components, including the antioxidant compounds in plant (Krokida & Philippopoulos 2006). During the freeze-drying process, ice crystals develop within the tissue matrix and removal of moisture content causes the tissue to become more brittle (Chan et al. 2009). The lower the moisture content, the greater the rupture of the cell structure, which may lead to higher solvent extraction efficiency of antioxidant compounds (Hossain et al. 2010; Ji et al. 2012; Shih et al. 2009). Investigating the effects of sample storage duration is recommended to maximize the determination of the antioxidant activity in the samples. Measuring the antioxidant properties of *C. trifolia* berries is especially useful in fruit jam production. Any processing method that maintains the level of antioxidant activities known for their health benefits will be of interest to the food and pharmaceutical industries.

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