Chemical composition and antioxidant activity of Torch Ginger (*Etlingera elatior*) flower extract

Aliaa binti Anzian[1], Sukor Rashidah[2], Nazamid Saari [1,2], Che Wan Nur Safraa binti Che Wan Sapawi[1] and Anis Shobirin binti Meor Hussin[1,3,*]

Abstract

Plant extracts with various bioactive compounds have gained considerable attention especially when used as functional ingredient. The aims of the present study were to profile the bioactive and volatile compounds in different maturity stages of torch ginger (*Etlingera elatior*) and also to study the effect of drying methods on bioactive compounds of torch ginger (*Etlingera elatior*) extract. The bioactive compounds in unopened and opened torch ginger were identified using Gas Chromatography-Mass spectrometry (GCMS). Unopened torch ginger flower was sun-dried with mid-day temperature ranged from 35–40°C for almost 27 hours, overnight oven drying at 40°C and the torch ginger also be lyophilized for 48 hours in a vacuum flask at 0.125 mbar and -50°C in a freeze-dryer till moisture content reached 10±2% in weight. The total phenols, total flavonoids and antioxidant activity of unopened torch ginger extract were determined by using UV-vis spectrophotometer. The most abundant compounds in torch ginger included á-pinene, decanal, and 1-dodecanol. Freeze-dried unopened torch ginger flower resulted the highest level of total phenols content (485.50±3.24mg GAE/100g), total flavonoids (61.26±14.87 mg GAE/100g), ferric reducing assay power (1943.50±84.15mg GAE/100g) and scavenging activity (89.24±1.24%) respectively. The extract from torch ginger flower could be potentially used as a new source of natural antioxidant in functional ingredient.

Keywords: Etlingera elatior inflorescence extract, drying methods, total phenolic content, total flavonoid content, antioxidant activity.

[1] Department of Food Technology, Faculty of Food Science & Technology, University Putra Malaysia, 43400, Malaysia

[2] Department of Food Science, Faculty of Food Science & Technology, University Putra Malaysia, 43400, Malaysia

[3] Halal Products Research Institute, University Putra Malaysia, Putra Infoport, 43400, Malaysia

Corresponding author, e-mail: shobirin@upm.edu.my

1. Introduction,

The past few decades has witnessed the use of several aromatic plant species for improving the healthy life style of humans the world over. The health benefits provided by these plants are attributed to the presence of various bioactive compounds. Among the various bioactive compounds (e.g., phenolic compounds like flavonoids, flavonols, etc.), plant extracts have gained considerable attention mainly due to their pharmaceutical and therapeutic potentials. (Edris,2007). Nowadays, the extracts which extracted from plant parts for instance leaves, flowers, barks, rhizomes, and fruits of aromatic plants have been discovered for their applications in food, drugs, and cosmetic products. (Tao *et al*.,2011 and Yao *et al*.,2010).

The Zingiberaceae family is recognized for aromatic plant species and comprises over 1400 species with 47 genera. (Pancharoen *et al*., 2000). The torch ginger plant (*Etlingera elatior*) belonging to the Zingiberaceae family, is one of the most popular aromatic plants found in the South-Asian regions of the world. This plant bears a bright pink colored inflorescence with a mild and pleasant aroma. The young inflorescence is used as a spice for food flavoring and in preparation of some traditional foods of Malaysia, such as “asam laksa,” “nasi kerabu,” and “nasi ulam”. (Chan *et al*., 2007; Wijekoon *et al*., 2010). The essential oil extracted from torch ginger plant parts (leaves, stems, flowers, and rhizomes) has been reported previously. (Jaafar *et al*., 2007). Additionally, pharmacological and toxicological activities of the methanolic extracts of the torch ginger flower have been reported. (Lachumy *et al*., 2010). However, to our knowledge no detailed reports are available on the antimicrobial activities of the essential oil or solvent extracts (results on water and ethanol are useful for food applications) of torch ginger inflorescence. Providing scientific details on torch ginger inflorescence assumes importance, as inflorescence is the part that is widely used for culinary purposes rather than the mature flowers. Thus, the purpose of the this study were to profile the bioactive and volatile compounds in different maturity stages of torch ginger (*Etlingera elatior*) and also to investigate the effect of drying methods on antioxidant activity, total phenolic and total flavonoid contents of torch ginger (*Etlingera elatior*) flower extract.

2. Materials and Methods

2.1 Chemicals and Instruments

2.1.1 Chemicals and reagents

Ascorbic acid, gallic acid, quercetin, sodium dodecyl sulphate, sodium nitrate and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Folin-Ciocalteu phenol reagent, potassium ferric cyanide, ferric (III) chloride hexahydrate ($FeCl_3$.6H2O), and sodium hydroxide (NaOH) were obtained from Merck (Darmstadt, Germany). Sodium

carbonate anhydrous, aluminium chloride and sodium acetate were purchased from Carlo Erba, UK. Hydrochloric acid, hexane, ethanol, methanol were purchased from Mallinckrodt, USA.

2.2 Plant Material Preparation

Inflorescences of the torch ginger (*Etlingera elatior*) plant were obtained from the same local supplier to avoid variation. Voucher specimens of torch ginger samples were identified by officer of Agricultural Conservatory Park, University Putra Malaysia, Selangor, Malaysia. The inflorescences used were fresh, unopened and opened of different maturity of growth with no apparent physical defects. The inflorescences were separated from stalks and stem and washed thoroughly under running water to remove dirt and their surface also cleaned cautiously to remove all the adhering debris. The excess water was drained and kept in cold storage at -20°C.

2.3 Drying Processes

The unopened torch ginger inflorescences were taken out of cold storage and thawed at room temperature. The torch ginger inflorescences were cut into a particle size about 3cm and subjected to three different drying methods (freeze-, oven-, and sun-drying) until moisture content reached 10±2% in weight. For each drying method, 150g of fresh torch ginger was used and flower pieces were spread out evenly on drying tray (41cm×21cm). In freeze-drying, flower samples were lyophilized overnight in a vacuum flask at 0.125 mbar and -105°C in a freezer-dryer (Model 7754511, Labconco Corporation, Kansas City, MO, USA). Oven-drying involved for 16h in oven (Memmert ULE 500) at 40°C. Torch ginger inflorescence were sun-dried for three days with about 27h of daylight, which mid-day temperature ranged from 35–40°C. After undergo different drying conditions, the dried torch ginger samples were ground to a fine powder size (below 500μm) using a commercial kitchen blender (Model BL 335, Waring, Selangor, Malaysia) and stored at room temperature in amber-colored glass bottles, covered with aluminium foil (to prevent direct exposure to light) until further analysis.

2.4 Solvent Extraction

Unopened torch ginger (*Etlingera elatior*) samples were extracted using method adopted by Chan *et al*. (2012); How and Yau (2011) and Siow and Hui (2013). Fine powdered flowers (1g) were transferred into a 100 ml extraction flask and extracted by 50 ml of ethanol (95% denatured ethanol) for 1 hour at room temperature. The mixture was swirled continuously for overnight (12h) on a hotplate with continuous magnetic stirring (at 150 rpm, 25±1°C). The resultant extract was filtered through filter paper Whatman No. 1 using vacuum filtration. Further, the filtrates were pooled and the solvent was evaporated in a rotary evaporator (Laborota 4000 Efficient Eco, Heidolph, Germany) at 40°C to obtain a final volume of

concentrate crude extract. Then, extract were dissolved in 50ml of ethanol were stored at 4°C for not more than 14 days for further analysis.

2.5 Determination of Phytochemical Compounds by GC-MS

The phytochemical components present in the unopened and opened torch ginger extracts were carried out as described by Lee *et al.* (2011) using Gas Chromatography – Mass Spectrometry Analysis (GC-MS). The sample (µg/ml) was diluted 10 times with hexane, 1µl of extract was injected into a column. The column equipped was 5MS fussed silica capillary column (30.0m×0.25mm×0.25µm, composed of 5% phenyl/ 95% dimethylpolysiloxane). Helium was the carrier gas, and a split ratio of 1/100 was used. The oven temperature was maintained at 60°C for 8 min. The temperature was then gradually raised at a rate of 3°C per min to 180°C and maintained at 180°C for 5 min. The temperature at the injection port was 250°C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 22 to 600Da). Total GC running time is 43 min.The chemical constituents were identified by matching their mass spectra with those stored in the spectrometer database using the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998), using retention indices as a preselection routine (Alencar *et al*., 1984a, 1990b). The name, molecular weight and structure of the components of the test extracts were ascertained.

2.6 Analysis of Antioxidant Properties

2.6.1 Determination of DPPH Radicals Scavenging Activity

Free radical scavenging (FRS) activity of unopened torch ginger flowers were measured in terms of hydrogen-donating or radical scavenging ability, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Chan *et al.* (2012) and How and Yau (2011). 0.2 mM DPPH solution was prepared by dissolving 0.0078g into 100 ml methanol. 1g of extract was added to 50ml of ethanol (20mg/ml). A serial dilution of extracts (20, 10, 5 and 2.5 mg/ml) were prepared. 50 µl of sample aliquot was added into 195 µl of DPPH in methanol. The mixture was shaken with vortex and incubated in the dark for 30 minutes. The absorbance of the solution was measured at 517 nm using a microplate reader (ELISA GENESYS 20 Ultra Visible Spectrophotometer microplate reader, Thermo Scientific, USA). All spectrophotometric measurements were using distilled water as blank. For the negative control, 1ml of methanol was used instead of the plant extracts. This assay was carried out triplicate. The scavenging ability percentage of the torch ginger extracts was expressed using the following equation:

$$\text{Scavenging ability (\%)} = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100\%$$

Where $Abs_{control}$ is the absorbance of DPPH solution in methanol without sample, and Abs_{sample} is the absorbance of DPPH solution in methanol with sample (after 30 minutes incubation).

The free radical scavenging activity of unopened torch ginger flower was calculated as IC_{50} (The concentration of extracts necessary to reduce the absorbance of DPPH radicals by 50%). IC_{50} of extracts was obtained from the plot of scavenging ability (%) against extract concentration (mg/ml). Ascorbic acid (AA) was used as a standard.

2.6.2 Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The ferric reducing antioxidant power of the extracts was determined in this study by reacting the unopened torch ginger flower extract (1 ml in triplicate) with distilled water (5 ml), then adding 1 M HCl (1.5 ml) and 1% potassium ferricyanide (1.5 ml). Then sodium dodecyl sulphate (SDS, 0.5 ml) and 0.5 ml of 0.2% ferric chloride ($FeCl_3$) were added, and the entire solution was incubated at 50 °C for 20min to complete the reaction. Ferrous sulphate was used as the standard and the absorbance was measured using a spectrophotometer at 700 nm. This assay was carried out in triplicate. Solutions of known ferrous sulphate ($FeSO_4.7H_2O$) concentrations in the range of (0.05–0.25 mg/ml) FRP of extracts was expressed as ferrous sulphate equivalents (GAE) per 100g of sample. The calibration equation for ferrous sulphate was y=4.884x + 0.3834 (R^2= 0.983), where y is the absorbance and x is the ferrous suphate concentration in mg/ml.

2.6.3 Determination of Total Phenolic Content

Total Phenolic Content (TPC) of extracts was determined using the Folin-Ciocalteu (FC) method according to Chan *et al*. (2012) with some modification. Extracts (20 µl in triplicate) were introduced into 96-well microplate (330 µl scale) followed by the addition of 100 µl of FC reagent (10 times dilution). Subsequently, sodium carbonate solution (80 µl, 7.5% w/v) was then added to form an alkaline environment for chemical reaction. The 96-well microplate was covered and mix thoroughly for approximately 5 seconds with gentle shake. The mixture then incubated at room temperature in the dark for 2h. The absorbance was measured against the blank reagent at 765 nm by a microplate reader after a programmed 30 s shake cycle. The blank solution was prepared by replacing 20 µl sample extracts with 20 µl of distilled water. This assay was carried out in triplicate. Gallic acid was used as the standard for the calibration curve and TPC was expressed as gallic acid equivalent (GAE) in mg per 100g of sample.

The calibration equation for gallic acid was y=10.689x + 0.1111 (R^2= 0.9962),

where y is the absorbance and x is the GA concentration in mg/ml.

2.6.4 Determination of Total Flavonoid Content

The total flavonoid content was determined based on aluminium chloride colorimetric assay according to a modified protocol developed by Chen and Li (2007). The mixture of torch ginger extract (25 µl in triplicate), distilled water (125 µl) and 10 µl of 5% sodium nitrate were added to 96- wellplate. After 6 minutes, 15 µl of 10% aluminium chloride solution was added. The mixture was allowed to stand for 5 minutes at room temperature. 50 µl of 1M sodium hydroxide was added to the mixture and shaken for 1 minute. The mixtures were incubated at room temperature for 10 minutes. The absorbance of the mixture was measured using a microplate reader at 415 nm versus sample blank of 25 µl of the respective extracts with 125 µl of distilled water. This assay was carried out in triplicate. A calibration curve was made by absorbance and the concentration of quercetin. The results of TFC were expressed as mg quercetin equivalents (QE) per 100g of sample.

The calibration equation for quercetin was $y=0.4645x + 0.0543$ ($R^2= 0.9836$),

where y is the absorbance and x is the QE concentration in mg/ml.

All experiments were done in triplicate (n=3) and results were expressed as means ± standard deviation (SD) to verify the statistical significance of all parameters. Analysis of variance (ANOVA) was performed using Minitab software to determine the efficiency of the drying methods for extraction as well as establish the differences in antioxidant activity and the content of phenolic, and flavonoid. A p-value of less than 0.05 was considered to be statistically significant ($p<0.05$).

3. Results and Discussion,

3.1.1 Plant description

Etlingera elatior (family: Zingiberaceae) is a perennial herbal plant (height of 3.6 to 4.7 m) found growing abundantly in parts of Malaysia, Indonesia, Vietnam, Sri Lanka, and Thailand. The flower (bud or inflorescence) is used both ornamentally and as a spice for culinary use. Rhizome and flowers of this plant are extensively used as a natural ingredient in cosmetics (as an ingredient of soap, shampoo, perfume) and also as a therapeutic agent for treating common ailments. Fruits of the torch ginger plant are traditionally used to treat ear ache, while leaves find use to clean wounds and to remove body odour (Chan *et al*., 2007). Flowers and the mature inflorescence of torch ginger are used to prepare such popular dishes as *asam laksa, nasi kerabu, nasi ulam* (in Malaysia), *arisk ikan mas* (in North Sumatra, Indonesia), and *sayur asam* (in Thailand) (Lachumy *et al*., 2010;Wijekoon *et al*., 2011). Torch ginger inflorescence is reported to possess strong antioxidant activities (Wijekoon *et al*., 2011).

3.1.2 Chemical composition

The chemical composition of unopened and opened torch ginger (*Etlingera elatior*) flower extracts studied by GC-MS are presented in Tables 1 and 2. Total ionic chromatograms of both unopened and opened torch ginger (*E. elatior*) flower extracts are presented in Figure 1 and 2. In this study, a total of 35 compounds with different relative area percentage were characterized in both flower extracts. The most abundant components in the unopened torch ginger (*E. elatior*) included Z-11-Pentadecenol (39.57%), 1-Dodecanol (12.16%), à-Pinene (9.68%), Dodecanal (9.24%), Octane (6.09%), Dodecanoic acid (4.88%), 1-Hexadecanol (2.63%), Lauryl acetate (2.25%), Caryophyllene (1.73%), Tetradecanoic acid (0.94%), 1-Tetradecyl acetate (0.62%), Decanal (0.40%), l-(+)-Ascorbic acid (0.36%) and Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl (0.24%) in Table 1.

Table 1 Compounds tentatively identified in the unopened torch ginger (*Etlingera elatior*) inflorescence

Compound no.	RT[a]	Similarity (%)	Compound[b]	RC[c] (%)	Molecular weight
1	6.53	51.83	Octane	6.09	114
2	9.92	8.43	à-Pinene	9.68	136
3	15.86	13.31	Decanal	0.40	156
4	16.11	12.46	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	0.24	150
5	19.66	52.26	Dodecanal	9.24	184
6	20.17	28.36	Caryophyllene	1.73	204
7	20.76	9.31	1-Dodecanol	12.16	186
8	22.23	85.42	Dodecanoic acid	4.88	200
9	22.90	30.15	Lauryl acetate	2.25	228
10	23.98	9.38	1-Hexadecanol	2.63	242
11	25.18	61.10	Tetradecanoic acid	0.94	228
12	25.86	17.64	1-Tetradecyl acetate	0.62	256
13	28.04	41.33	l-(+)-Ascorbic acid	0.36	652
14	31.29	7.43	Oleic acid, eicosyl ester	0.42	562
15	32.43	17.83	2,3,3',4'-tetramethoxy-5-(3-methoxyprop-1-enly)stilbene	0.34	370
16	35.61	17.60	Z-11-Pentadecenol	39.57	226

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Note: [a] RT' Retention Time (min).

[b] Compound are listed in order of their relative area percentage.

[c] Relative area percentage (peak area relative to the total peak area percentage).

Table 2 Compounds tentatively identified in the opened torch ginger (*Etlingera elatior*) inflorescence

Compound no.	RT[a]	Similarity (%)	Compound[b]	RC[c] (%)	Molecular weight
1	6.52	41.10	Octane	1.52	114
2	9.92	5.81	à-Pinene	7.93	136
3	15.86	27.53	Decanal	0.48	156
4	16.11	24.77	Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl	0.23	150
5	17.13	13.70	1-Decanol	0.44	158
6	17.55	34.56	2-Undecanone	0.18	170
7	17.81	26.63	Undecanal	0.35	170
8	19.67	58.48	Dodecanal	11.47	184
9	20.17	26.30	Caryophyllene	2.46	204
10	20.78	6.32	1-Dodecanol	21.28	186
11	22.29	77.74	Dodecanoic acid	6.30	200
12	22.90	33.78	Lauryl acetate	2.89	228
13	23.99	7.78	1-Hexadecanol	5.59	242
14	25.22	79.81	Tetradecanoic acid	1.28	228
15	25.86	25.39	1-Tetradecyl acetate	1.05	256
16	28.10	39.71	l-(+)-Ascorbic acid	2.21	652
17	28.22	26.99	Hexadecanoic acid,	0.22	568
18	35.78	20.06	Z-11-Pentadecenol	23.27	226
18 out of 35					

Note: [a] RT' Retention Time (min).

[b] Compound are listed in order of their relative area percentage.

[c] Relative area percentage (peak area relative to the total peak area percentage).

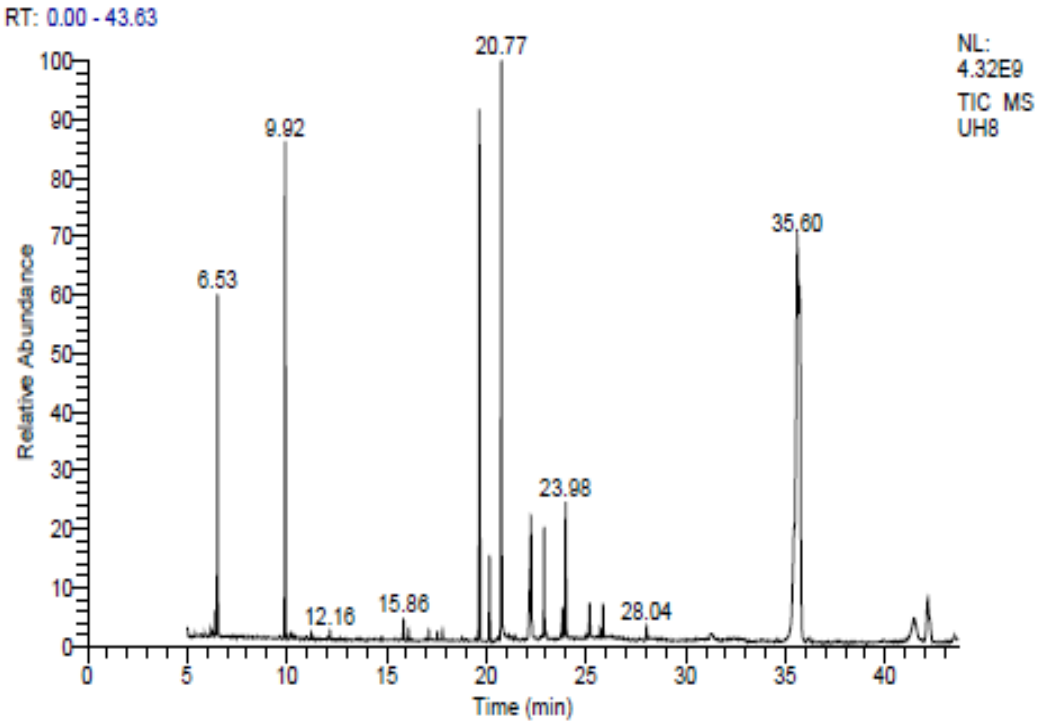

Figure 1 GC-MS Chromatogram of unopened torch ginger (*Etlingera elatior*) inflorescence extract

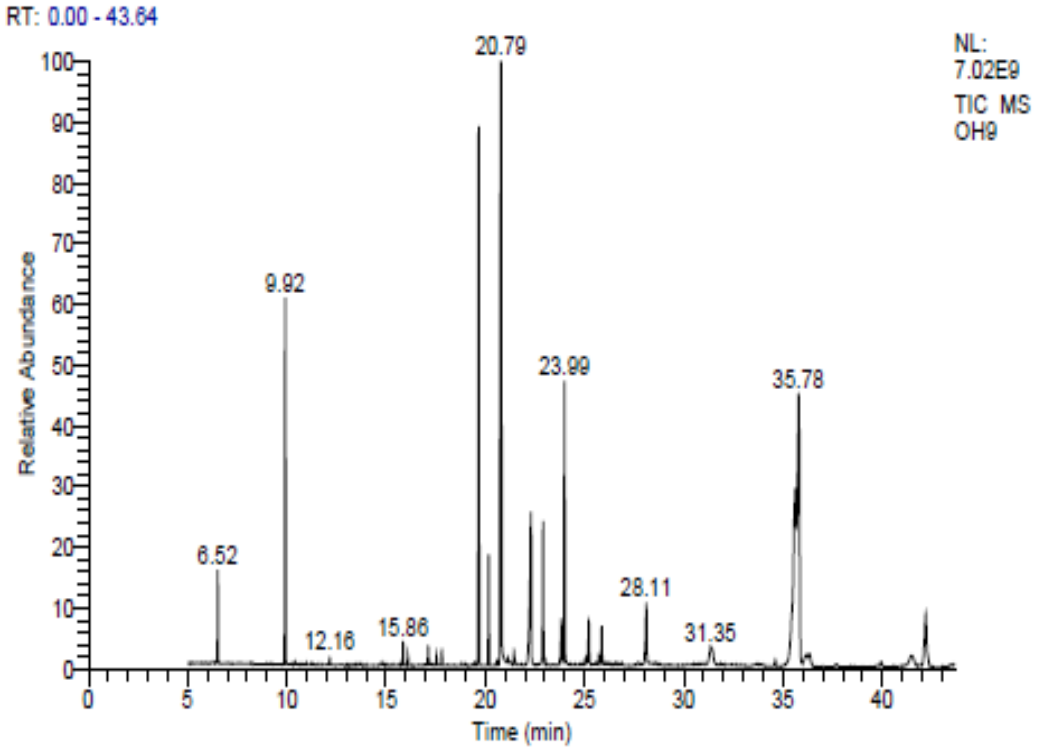

Figure 2 GC-MS Chromatogram of opened torch ginger (*Etlingera elatior*) inflorescence extract

While in Table 2 , Z-11-Pentadecenol (23.27%), 1-Dodecanol (21.28%), Dodecanal (11.47%), à-Pinene (7.93%), Dodecanoic acid (6.30%), 1-Hexadecanol (5.59%), Lauryl acetate (2.89%), Caryophyllene (2.46%), Tetradecanoic acid (1.28%), 1-Tetradecyl acetate (1.05%), Decanal (0.48%), and l-(+)-Ascorbic acid (2.21%) were the major compounds in the opened torch ginger (*E. elatior*) flower extracts . Undecanal (0.35%) and 2-Undecanone (0.18%) also have been identified.

There are many reports on the extract composition of inflorescences of torch ginger (*E. elatior*). Twenty five compounds were identified previously by Arbaayah (2008) and Jaafar *et al*. (2007) with 1-dodecanol as the major component represent 61.59 and 40.32% of overall extracts percentage respectively. Zoghbi *et al*. (2005) found only 15 compounds from the inflorescences with 42.5% of 1-dodecanol as major component. Recently, Abdelwahab *et al.* (2010) found 73 compounds from the leaves of this species. All the previous studies indicated that 1-dodecanol as the major component in torch ginger (*E.elatior*) inflorescence extract. This percentage difference of 1-dodecanol might be due to different extraction techniques, genetic factors, different chemotypes and the nutritional status of the plants as well as other environmental factors. The current study demonstrated a higher percentage of major components in opened torch ginger (*E.elatior*) extract compared to unopened torch ginger. Comparison of the results showed that the different maturity of plants had an effect on the percentage of the main compounds. Increased concentrations of various volatile substances in more matured plants have been observed for numerous spices and are probably caused by the breakdown of glycosylated forms, dehydration reactions, or oxidation reaction (Baritaux *et al*., 1992; Bartley and Jacobs, 2000) or due to the rupture of the matured plant cells in which the volatiles are stored. Some volatile compounds could arise from the dehydration of oxygenated compounds, such as acetic acid, dodecyl ester, 1-tetradecanol, and n-decanoic acid. In both unopened and opened torch ginger (*E.elatior*) flower extract consist of 1-dodecanol as the most abundant alcohol and followed by hexadecen-1-ol, trans-9. According to Wijekoon *et al*., (2015), these two alcohols have been reported to exhibit rich antimicrobial activities against microbial pathogens.

3.2 Antioxidant analysis

Results obtained for antioxidant activity (percent of DPPH radical scavenging activity and FRAP assay) and antioxidant compound (Total Phenolic and Flavonoid content) only from unopened torch ginger (*E. elatior*) flower extracts due to limited accessibility of opened torch ginger (*E. elatior*) flower. With regard, the fresh unopened torch ginger (*E. elatior*) flowers were undergone freeze, oven and sun dried respectively.

3.2.1 Percent of DPPH radical-scavenging activity

DPPH is a stable organic nitrogen radical containing an unpaired electron. It is a purple chromogen giving maximum UV-visible absorption at 515–520 nm. DPPH assay is widely used in the determination of free radical scavenging activity of natural antioxidants, mainly due to its simplicity and high sensitivity. In this assay, an antioxidant donates hydrogen or electron which is then accepted by the DPPH radicals (Moon and Shibamoto, 2009). Antioxidants such as ascorbic acid, à-tocopherol and polyphenols are able to act as reducing agents to DPPH radical which will delocalize the spare electron in DPPH molecule causing to fade in colour (Molyneux, 2004).

In the present study, ascorbic acid was used as a standard at various concentrations (0–0.20 mg/ml). Likewise, unopened torch ginger flower extract from freeze, oven and sun-dried were prepared at various concentration (2.50, 5.00, 10.0 and 20.0 mg/ml). At the concentration of 20 mg/ml, results of DPPH radical scavenging activity showed that freeze dried of unopened torch ginger (*E. elatior*) flower extract had the highest radical scavenging activity (89.24%) as compared to oven and sun dried extracts with (82.73%) and (56.22%) respectively. Based on the results shown in Table 3, it was evident that the percentage of the radical scavenging activity values increased with the corresponding reduction in the concentration of ethanol (used as solvent extraction). The higher the percentage of DPPH radical scavenging activity demonstrated, the better the antioxidant activity. IC_{50} (Inhibition concentration) to reduce 50% of DPPH radical had been calculated from a plotted graph (percentage of scavenging activity versus sample concentration). The lowest concentration of the extract needed to reduce 50% of the DPPH radical indicates the highest antioxidant activity. IC_{50} values were also summarized in Table 3, contradictorily sun dried of unopened torch ginger flowers extract had the highest IC_{50} value with a concentration of 18.32 mg/ml followed by oven and freeze dried extracts with IC_{50} values of 12.59 mg/ml and 9.52 mg/ml. Thus, the present study found that freeze-dried unopened torch ginger flower extract were better radical scavenger as compared to extract which undergo oven and sun drying method.

This probably due to no thermal degradation in freeze drying and the freeze drying method does not allow the process degradative enzymes to function. Furthermore, freeze drying is known to have high extraction efficiency because ice crystals formed within the plant matrix can rupture cell structure, which allows exit of cellular components and access of solvent, and consequently better extraction (Asami *et al*., 2003).

Previous study by Wijekoon *et al*., (2011) found that 50% acetone extract of torch ginger inflorescence showed highest percentage of DPPH radical scavenging activity (94.9%). This has been opined that with the change of solvent polarity, vapour pressure and viscosity,

the type of antioxidant compound being dissolved in the solvent also varies. Solvents with low viscosity have low density and high diffusivity that allows them to easily diffuse into the pores of the plant materials to leach out the bioactive constituents (Naczk and Shahidi, 2006).

Table 3 DPPH radical scavenging effect (%) of samples and IC_{50}

Drying	Concentration (mg/ml)	Scavenging activity (%)	IC_{50} (mg extract/ml)
Freeze	20	89.24 ± 1.24	9.52
	10	65.75 ± 19.78	
	5	30.90 ± 10.50	
	2.5	20.58 ± 14.18	
Oven	20	82.73 ± 1.24	12.59
	10	40.62 ± 19.78	
	5	15.02 ± 10.50	
	2.5	0.89 ± 14.18	
Sun	20	56.22 ± 10.44	18.32
	10	19.19 ± 1.24	
	5	13.63 ± 10.72	
	2.5	3.15 ± 17.8	

Note: *IC_{50} values represent 50% of the radicals scavenged by the test samples. A lower IC_{50} value has higher antioxidant activity.

3.2.2 Ferric Reducing Antioxidant Power (FRAP)

FRAP assay is basically involved in electron transfer reaction. At lower pH, reduction of ferric (Fe^{3+}) tripyridly triazine complex to ferrous form (Fe^{2+}) occurs to form deep blue colour which can be measured using UV-spectrophotometer micro plate reader at 700 nm. The change in absorbance is directly related to the concentration of antioxidant present in the reaction mixture. Hence, the higher the FRAP values, the higher the antioxidant activity obtained. According to Table 4, results of ANOVA analysis indicated that there was significant difference (p<0.05) between freeze dried unopened torch ginger (*E.elatior*) flowers which showed significantly highest values in FRAP (1943.01±84.15 mg Fe^{2+}/100g) compared to oven (1433.18±18.00 mg Fe^{2+}/100g) and sun dried (707.68±43.16 mg Fe^{2+}/100g) torch ginger flower extracts. Sun drying of unopened torch ginger (*E.elatior*) flower resulted in significant losses in FRAP value could be due to enzymatic degradation as the process was carried out under sunlight and took several days for samples to dry. Many studies have reported losses in

antioxidant activity of plant samples following thermal treatments including oven and sun drying which have been attributed to degradative enzymes, thermal degradation of phytochemicals, and loss of antioxidant enzyme activities (Lim & Murtijaya, 2007). Decline in antioxidant activity are often accompanied by loss of other bioactive properties (Roy *et al*., 2007).

3.2.3 Total Phenolic Content

Phenolic compounds in plants are known to act as free radical scavengers and it has been opined that the antioxidant activity of the most plant produce is mainly due to the presence of phenolic compounds (Skerget *et al*., 2005). Basically, antioxidant mechanism of polyphenolic compounds is based on their hydrogen donating and metal ion chelating abilities (Lee *et al*., 2004; Jacobo-Velazquez and Cisneros-Zevallos, 2009).

The total phenolic content (TPC) values of unopened torch ginger (*E.elatior*) flower extract summarized in Table 4 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). Results of ANOVA analysis indicated that there was significant difference ($p<0.05$) between freeze dried torch ginger flowers in all drying methods studied. However, this study revealed that there is no significant difference in TPC values for both oven and sun drying method.

Overall, considering all three different drying methods, freeze dried (485.50±3.24 mg GAE/100g) of unopened torch ginger flowers was found to be the most effective for phenol extraction with highest total phenolic content and sun dried (339.40±24.93 mg GAE/100g) was the least effective drying method for phenol extraction. This finding was in agreement with the study of Chang *et al*. (2006) whereby had reported that there was significant increases 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 total phenolic content.

3.2.3 Total Flavonoid Content

Flavonoids are the most common and widely distributed important single group of phenols present in plants that are highly effective as antioxidants (Yanishlieva-Maslarova, 2001). Flavonoids can inhibit metal-initiated lipid oxidation by forming complexes with metal ions (Lee *et al*., 2004). The potential antioxidant activity of flavonoids is associated with the

chemical structures with o-diphenolic group, a 2–3 double bond conjugated with the 4-oxo function and hydroxyl groups in positions 3 and 5 (Bravo, 1998).

Comparisons of TFC between freeze, oven and sun dried torch ginger flower were presented in Table 4, indicated that there was significant difference (p<0.05) between freeze, oven and sun dried unopened torch ginger flower extracts. Sample undergone freeze drying showed highest amount (61.26±14.87 mg QE/100g) of total flavonoids content followed by oven (51.01±64.74 mg QE/100g) and sun dried (17.61±9.67 mg QE/100g) respectively. With the increasing concentration of organic solvent, a corresponding significant decrease in the total flavonoids was observed. These results highlight that the yield of extraction of phenolic and flavonoids compounds is dependent on the drying treatment being used prior to extraction.

Drying is the most common and fundamental method for post-harvest preservation of medicinal plants because it allows for the quick conservation of the medicinal qualities of the plant material in an uncomplicated manner (Muller and Heindl, 2006). Tanko *et al*., (2005) reported that freshly harvested plants occupy large volumes and pose difficulty in transportation and storage. Hence, the drying of medicinal plants is necessary for handling and preservation purposes, but drying protocols must be designed such as they do not result in a decrease in phytochemical concentrations (Tanko *et al*., 2005). Drying the plant materials results in increase of oil yields (Faridah *et al*., 2010) and accelerates distillation, by improving the heat transfer (Whish and Willams, 1998). Other advantages are the reduction of microbial growth and the inhibition of some biochemical reactions in the dried material (Baritaux *et al*., 1992; Combrinck *et al*., 2006).

Table 4 Results of antioxidant content in Unopened Torch Ginger inflorescence (UTG)

Drying method	Total Phenolic Content (mg GAE/100g)	Total Flavonoid Content (mg QE/100g)	Ferric Reducing Power Assay (mg Fe^{2+}/100g)
Freeze	485.50 ± 3.24^{a}	61.26 ± 14.87^{a}	1943.01 ± 84.15^{a}
Oven	356.55 ± 9.78^{b}	51.01 ± 64.74^{b}	1433.18 ± 18.00^{b}
Sun	339.40 ± 24.93^{b}	17.61 ± 9.67^{c}	707.68 ± 43.16^{c}

Note: Values were expressed as mean ± standard deviation (n=3)

Means with different letters in column were significantly different at the level of p <0.05.

4. Conclusion,

This study shows that, freeze drying is superior to other drying methods in preserving the antioxidant activity, total phenolic and flavonoid content of unopened torch ginger flower. While, sun drying resulted in significant declines in DPPH, FRAP assay, TPC and TFC. Due to high operation cost, freeze drying can be replaced by oven drying but the conditions for oven drying must be optimized to yield high-value antioxidant capacity from torch ginger flower. From GCMS analysis, it is also envisaged that the torch ginger flowers might possess high antimicrobial activities with promising potential to be used in food industries as a natural preservative as well.

Acknowledgements

The authors are grateful to Universiti Putra Malaysia and the Ministry of Higher Education, Malaysia for financial support.

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