Effects of Different Drying Methods on Antioxidant Properties of Malaysian Ginger

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Abstract. Malaysian ginger is one the sought herbs that contains a lot of beneficial properties that could contribute to health. However, most usage of ginger from its fresh form which is prone to microorganisms’ spoilage. Therefore, effects of shade, sun, oven, vacuum oven, and freeze drying on phytochemical contents, ferric reducing antioxidant power (FRAP), phosphomolybdenum assays, 2,2′azinobis (3-ethylbenzothiozoline-6-sulfonic acid) disodium salt (ABTS•⁻), 2,2-diphenyl-1-picryl-hydrazyl (DPPH•), hydroxyl (OH•) radical and metal chelating properties of Halia Bara were studied. Dried and fresh ginger crude extracts were extracted with ethanol. The freeze-dried extract had highest level of total phenolic of about 20.07 mg GAE/g dry extract as compared to fresh ginger extract at 10.52 mg GAE/g dry extract. For antioxidant activity of FRAP and phosphomolybdenum, sun-dried extract exhibited the highest values with increase of 3.95-fold and 4.29-fold from fresh ginger extract, respectively. In scavenging ABTS•⁻ radical, sun-dried extract also exhibited the highest values with increase of 2.07-fold from fresh ginger extract. Sun-dried extracts also had the lowest IC50 of 14.69 µg/ml. The ascorbic acid of ginger types was below 1.5 mg AA/g extract. Sun-dried ginger extract exhibited most significant antioxidant potential and as free radical scavengers.

1. Introduction
Antioxidants play an important role in humans system as defense mechanism by stabilizing or converting the free radicals to less reactive molecules before attacking the cells [1,2]. Antioxidants is defined as a substance which prevent oxidation of process [3], inhibit radical chain reactions, scavenge free radicals, and retard process of lipid peroxidation [4]. In an oxidative stressed conditions, the amount of antioxidant present may be insufficient to neutralize or balance the free radical generated [1]. Hence, there has been an increased interest in natural antioxidants from plants antioxidant rich dietary materials. Several plants and their active constituents are rich source of antioxidant and play a crucial role in prevention of degenerative diseases [5]. These natural antioxidants are also known as redox-active secondary metabolites of plants [3,6].

Ginger, Zingiber officinale Roscoe contains a large number of antioxidants and has the capability to reduce oxidation of lipid and inhibit pathogenesis diseases. Previous study reported that some phenolic substances and bioactive constituents present in ginger have shown a vital effect in anti-oxidative and anti-inflammatory properties and exert anti-mutagenic and anti-carcinogenic activities [3,5,7,8]. Halia Bara is popular and sought of ginger types in Malaysian and neighbouring countries as main seasoning
in diet and also for medicinal reasons. Recent practice of using sun-dried ginger for suppressing illnesses due to free radicals in local community prompted studies on drying effects of ginger [9,10]. Sun drying is a drying source that may be preferred to other drying methods because it is abundant, unlimited, renewable, inexpensive, non-pollutant and environmentally friendly [11]. Malaysia weather benefits from a tropical climate with uniform temperature, high humidity and sunny has prompted this study.

In general, drying is a postharvest processing method meant to preserve and extend the shelf life of food products. Dried products would inhibit microbial growth and prevent changes in biochemical components. Although drying process is known to give adverse effects on physical properties, nutrients and also on the antioxidant activity [7,12], information on effect of different drying methods on various antioxidant properties and activities of Malaysian ginger are not available. Henceforth, phytochemical composition in terms of antioxidant properties and activities of ethanolic extracted dried and fresh Malaysian ginger types were studied.

2. Research Method

2.1 Drying and Extraction Procedures

Halia Bara was obtained in the farm area of Tendong, Pasir Mas, Kelantan and was subjected to different methods of drying [13]. The dried ginger then was extracted using maceration techniques [13].

2.2 Determination of phenolic content, FRAP, TAA and DPPH• inhibition

The phenolic content and antioxidant activities of ginger extracts (FRAP, scavenging of ABTS•+ and DPPH• inhibition) were reported somewhere [13].

2.3 Ascorbic acid content

Ascorbic acid of ginger was determined following the method described by Klein and Perry [14]. 150 mg of crude extract was re-extracted with 10 ml (1% w/v) metaphosphoric acid for 45 min at room temperature and filtered using Whatman filter paper (No. 4). 1 ml of filtrate was mixed with 9 ml (0.005% w/v) of 2,6-dichlorophenolindophenol (DCPIP), and the absorbance was measured immediately at 515 nm against a blank. The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid and the results were expressed in mg ascorbic acid (AA)/g dry extract of ginger.

2.4 Phosphomolybdenum assay

The phosphomolybdenum method is based on the reduction phosphate-molybdenum (VI) to phosphate-molybdenum (V) by the antioxidant at acidic pH. The phosphomolybdenum assay of ginger extract was evaluated by the method of Prieto et al. [15]. 100 µl of extract was added with 3.6 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in test tubes. The test tubes were capped and incubated at 95 °C in water bath for 30 min. The mixtures were cooled to room temperature and the absorbance of each solution was measured at 695 nm against the reagent blank. The results were reported mean values expressed in grams of ascorbic acid equivalents per 100-gram sample (AEAC).

2.5 Free radical scavenging activity on hydroxyl OH•

The scavenging activity of ginger extracts on hydroxyl (OH•) radical was measured following Klein’s method [16] with some modifications. The extracts at concentrations from 0 to 100 µg/ml were added with 1 ml of iron-ethylenediamine- tetraacetic acid (Fe-EDTA) solution (0.13% w/v ferrous ammonium sulfate and 0.26% w/v EDTA), 0.5 ml of EDTA solution (0.018% w/v) and 1 ml of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22% w/v) and was incubated at 88°C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1 ml of ice-cold trichloroacetic acid (TCA) (17.5% w/v). 3 ml of Nash reagent (7.6g of ammonium acetate, 0.3 ml of glacial acetic acid and 0.2 ml of acetyl acetone were mixed and made up to 100 ml with distilled water) was added and measured at 412 nm against
reagent blank. The reaction mixture without sample was used as control. The percentage hydroxyl radical scavenging activity was expressed as the inhibition of free radicals by the samples Equation (1).

\[
\text{OH}^\cdot \text{scavenging activity (\%)} = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{\text{Control absorbance}} \times 100\% \tag{1}
\]

2.6 Determination of metal chelating potential of ginger extracts
The chelating of ferrous ions by ginger extract was estimated by method of Dinis et al. [17] with minor modification. The extract samples (100 µl) were added to a solution of 2mM ferrous chloride (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and made up to 5 mL with distilled water. The mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g dry extract.

2.7 Statistical Analysis
The data obtained were presented as means of 3 replicate determinations ±standard deviation (SD). The data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Tukey comparison test (P < 0.05) using statistical Minitab 16 software (Minitab Inc., State College, PA, USA).

3. Results and Discussion
3.1 Recovery percent, total phenolic and ascorbic acid contents
Table 1 shows the extract yields, total phenolic and ascorbic acid contents of fresh and dried ginger prepared by different drying methods. The extract yield of *Halia Bara* ranged from 2.57% to 9.37%. The maximum extract yield was obtained from sun drying at 9.37%. Extract yield from fresh extract was the lowest with 2.57%.

Phenolic extracts are considered as secondary metabolites components. They are directly responsible for antioxidant activities. Freeze-dried extract of *Halia Bara* had highest level of total phenolic with 20.07 mg GAE/g extract. The phenolic values of freeze-dried extract was 1.9 fold significantly higher (P < 0.05) than fresh extracts. Vitamin C is a powerful water-soluble antioxidant found in many dietary plants, which plays an important role in the inhibition of free radicals [18]. Generally, ascorbic acid blocks ‘free radicals’ before they attack cellular membrane by acting as reducing agent by electron donation [7]. In this study, ascorbic acid content in both gingers extract were less than 1.5 mg AA/g extract, which is approximately less than 0.15g/100g (recalculated). Other studies claimed that ascorbic acid content in ginger is not available [7] or very low at 0.91 mmol/100g and 1.83 mmol/100g low in white and red ginger, respectively [19]. To the best of our finding, result shows *Halia Bara* contain less amount of ascorbic acid.

| Sample         | Extract yield (%) | Total phenolic (mg GAE/g dry extract) | Ascorbic acid (mg AA/g dry extract) |
|----------------|------------------|--------------------------------------|------------------------------------|
| Fresh          | 2.57             | 10.526 ± 0.21d                       | 0.690 ± 0.01bc                     |
| Shade-Dried    | 6.84             | 16.438 ± 0.17c                       | 0.579 ± 0.10c                      |
| Sun-Dried      | 9.37             | 18.941 ± 0.29b                       | 0.813 ± 0.04b                      |
| Oven-Dried     | 6.85             | 16.083 ± 0.52c                       | 1.137 ± 0.13a                      |
| Vacuum Oven-Dried | 6.56        | 19.533 ± 0.31ab                      | 0.719 ± 0.06bc                     |
| Freeze-Dried   | 8.86             | 20.068 ± 0.52a                       | 0.863 ± 0.02b                      |

Values are mean (n=3) ± standard deviation.
Mean values followed by different superscripts in a column are significantly different (P < 0.05).

3.2 Reduction power by FRAP and phosphomolybdenum assays
Table 2 shows the FRAP and phosphomolybdenum assay prepared by different drying methods. Sun-dried extract had highest ferric reducing antioxidant activity with 4033.1 mmol Fe (II)/g dry extract. FRAP values of sun-dried extracts was 3.95-fold significantly higher (P < 0.05) than fresh extracts. Sun-dried also exhibited a higher phosphomolybdenum reduction of 81.12 g AA/100 g dry extract. The phosphomolybdenum reductive ability was significantly increased (P < 0.05) 4.29-fold than fresh extracts. The reducing capacity of ginger extracts suggesting that *Halia Bara* may serve as significant electron donors in antioxidant activity.

Table 2. FRAP and phosphomolybdenum activity of *Halia Bara*

| Sample                | FRAP (mmol Fe(II) / g dry extract) | AEAC (g AA/100 g dry extract) |
|-----------------------|------------------------------------|--------------------------------|
| Fresh                 | 1021 ± 29.7d                       | 18.90 ± 0.56f                  |
| Shade-Dried           | 3229 ± 25.2c                       | 74.27 ± 2.68b                  |
| Sun-Dried             | 4033 ± 29.9a                       | 81.12 ± 0.58e                  |
| Oven-Dried            | 3584 ± 61.1b                       | 59.34 ± 1.56e                  |
| Vacuum-Oven-Dried     | 3174 ± 117.3c                      | 64.72 ± 1.51d                  |
| Freeze-Dried          | 3219 ± 72.5c                       | 69.47 ± 1.26c                  |

Values are mean (n=3) ± standard deviation.

3.3 Radical scavenging activities by ABTS•+, DPPH• and OH•
Table 3 shows that sun-dried had highest scavenging ABTS•+ activity at 1712 mmol equivalent Trolox/g dry extract. The TEAC values of sun-dried extracts was 2.07-fold significantly higher (P < 0.05) than fresh extracts. The increase of TEAC values is reflective to higher polyphenols content of the dried extracts. It has been reported that an increase in antioxidant activity after drying process has been attributed to the release of bound phenolic compounds as cellular constituents would breakdown and new compounds with enhanced antioxidant properties were formed [19].

Scavenging the DPPH• radical is another method to study the potential of radical scavengers. The antioxidant activity of plant extracts is reflected to phenolic components as their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [20]. Thus, DPPH• radical will be reduced by antioxidant analyte to α, α-diphenyl- β-picrylhydrazine (yellow), with respect to the degree of reduction. A lower concentration of ginger extract to decrease the initial concentration of DPPH• by 50% (IC50) indicates higher antioxidant activity. The best free radical scavenging activity was exerted by sun-dried extract with IC50 14.69 µg/ml. This was higher than the reference standard butylated hydroxyanisole (BHA) at 28.59 µg/ml. The fresh extracts ginger had high values of IC50 which possess lowest antioxidant activity. The increased DPPH activity of dried ginger extracts is related to an accumulation and increase of phenolic in dried substance [20,21].

OH• radical is one of the reactive free radicals in living systems that cause DNA blockage and lead to strand breakage. This can contribute to carcinogenesis, cytotoxicity and mutagenesis in living systems [22]. *Halia Bara* extracts at different concentrations were tested for hydroxyl radical scavenging activity. The percentage of OH• radical scavenging activity was not significant different for the different drying extracts (figure 1).
Table 3. ABTS⁺ and DPPH⁺ scavenging activities of *Halia Bara*

| Sample            | TEAC (mmol/g dry extract) | IC₅₀ of DPPH (µg/ml) |
|-------------------|---------------------------|----------------------|
| Fresh             | 829 ± 44.0ᵈ               | 65.82 ± 6.83ᵃ        |
| Shade-Dried       | 1569 ± 21.2ᵇᵈ            | 27.19 ± 0.54ᵇ        |
| Sun-Dried         | 1712 ± 1.7ᵃ              | 14.69 ± 2.34ᶜ        |
| Oven-Dried        | 1428 ± 51.8ᵇᶜ            | 27.97 ± 1.92ᵇ        |
| Vacuum Oven-Dried | 1419 ± 90.9ᵇᶜ            | 28.08 ± 0.29ᵇ        |
| Freeze-Dried      | 1336 ± 72.8ᶜ             | 28.59 ± 0.83ᵇ        |
| Standard          | BHA                       | 28.59 ± 0.10         |

Values are mean (n=3) ± standard deviation.
Mean values followed by different superscripts in a column are significantly different (P < 0.05)

3.4 Metal chelating ability
Iron is a redox active metal which tends to undergo redox cycling reactions such as Fenton reaction and generate many reactive oxygen species (ROS) which lead to lipid peroxidation, hydroperoxide decomposition, protein modification and other effects [18]. Chelation of iron by extract will avoid its participation in redox reaction and prevent subsequent oxidative stress. The binding of ferrous ion by fresh and dried ginger extracts were estimated by disrupting the Fe²⁺-ferrozine complex which results the decrease of red colour intensity of the complex. Colour reduction would indicate that the extracts could chelate irons and possesses metal chelating activity. Table 4 shows that all sample extracts had potential chelating ability on Fe²⁺ but not unanimous in trend. Chelating agents are effective as secondary antioxidants as they capable to reduce redox potential, thereby stabilizing the oxidized form of metal ion [12]. Significant differences of metal chelating activities were identified in the table 4.
Table 4. Metal chelating activity (mg EDTA/g dry extract)

| Sample          | Halia Bara |
|-----------------|------------|
| Fresh           | 32.23 ± 7.50<sup>ab</sup> |
| Shade-Dried     | 44.69 ± 0.27<sup>ab</sup> |
| Sun-Dried       | 45.47 ± 9.49<sup>a</sup>  |
| Oven-Dried      | 51.39 ± 7.91<sup>a</sup>  |
| Vacuum oven-Dried| 47.80 ± 3.06<sup>a</sup>  |
| Freeze-Dried    | 21.32 ± 15.29<sup>b</sup>|  

Values are mean (n=3) ± standard deviation.
Mean values followed by different superscripts in a column are significantly different (P < 0.05).

3.5 Correlation analysis
In this study, a significant positive correlation (P < 0.05) was found between total phenolic content (TPC) and antioxidant properties from ginger extracts as tabulated in table 5. Phenolic content of Halia Bara were highly correlated with FRAP, phosphomolybdenum (PM), ABTS•+ and DPPH•. This strong relationship was due to hydroxyl group of phenolic compounds which enable them to participate in redox reactions in living mechanism. Reducing power of ginger extract was also positively correlated with radical scavenging activity. Correlation of antioxidant properties with ascorbic acid (AA) and metal chelating were low.

Table 5. Correlation (R²) among phytochemical content, antioxidant and metal chelating activities of ginger extracts

| Phytochemical content | Reducing power | Radical scavenging | Metal chelating |
|-----------------------|----------------|--------------------|-----------------|
| Halia Bara            |                |                    |                 |
| TPC                   | 1              |                    |                 |
| AA                    | 0.137          | 1                  |                 |
| FRAP                  | 0.821          | 0.362              | 0.939           |
| PM                    | 0.867          | 0.085              | 0.938           |
| ABTS<sup>++</sup>     | 0.739          | 0.127              | 0.938           |
| DPPH•                 | 0.801          | 0.234              | 0.978           |
| Fe<sup>2+</sup> chelating activity | 0.052 | 0.164 | 0.356 | 0.437 | 0.332 |

4. Conclusion
Drying methods could enhance ginger’s antioxidant capacity and as free radical scavengers. Drying process might breakdown the cellular constituents, thus promoting the release of bound phenolic compounds from food matrix. Sun dried gingers exhibited high total phenolic contents with 18.94 mg GAE/g dry extract and antioxidant activities through measured of FRAP (4033 mmol Fe(II)/g dry extract), ABTS<sup>++</sup> (1712 mmol/g dry extract), DPPH• (14.69 µg/ml) and AEAC assay (81.12 g AA/100 g dry extract). Further work is on identification of composition and components of fresh and dried ginger extracts at molecular levels.

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