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Antioxidant Capacity and Total Phenolic Content of Fresh, Oven-Dried and Stir-Fried Tamarind Leaves

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Abstract

The aim of this study was to compare the antioxidant capacity and total phenolic content as well as the chemical groups of fresh, oven-dried and stir-fried tamarind (*Tamarindus indica* L.) leaves. Methanol was used for extraction of fresh, oven-dried and stir-fried tamarind leaves. The stir-fried leaves were prepared using medium heat for 10 minutes prior to extraction and chemical analysis while dried leaves were obtained by oven drying at 60°C for 3 hours. The stir-fried leaves had significantly highest total phenolic content (TPC) (139.87 mg/g) and percentage DPPH radical-scavenging inhibition (69.92%) while the fresh leaves had the lowest TPC (39.31 mg/g) and antioxidant capacity (16.46%). The FTIR spectral data suggest that the heat treatment increased the amine groups as well as the antioxidant capacity of the tamarind leaves. To increase the antioxidant capacity, the tamarind leaves should be prepared in a stir-frying process.

Article History

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Keywords

Antioxidant capacity;
phenolic content;
tamarind;
stir-fried;
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Introduction

Tamarind (*Tamarindus indica* L.) is a tropical tree used primarily for its fruits as a seasoning, spice and medicine beverage. Besides, it has edible leaves rich in fat, fiber, vitamins, proteins and flavonoids[1]. Tamarinds leaves have a high content of polyphenols and are employed as antimicrobial agents[2]. High antioxidant capacity of tamarind dried leaves has been reported by[1] implies the tamarind could offer a natural alternative source of antioxidants. The air-dried tamarind leaves had phenolic content 50 mg GAE/g dried weight and achieved as high as 80% inhibition of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals[1]. Polyphenolic compounds are the main phytochemical compounds for the antioxidant capacity in herbs[3,4]. Higher total phenolic content contribute to higher total antioxidant capacity. Sufficient amounts of antioxidants are vital to limit the buildup of free radicals and oxidative damage in the body.

The processing of herbal drugs is a typical practice before applying for medicinal use in clinic. Various methods can be used to prepare the same medicinal

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plant, such as stir-frying, baking with vinegar, and water steaming[5]. The processing of plant, particularly medicinal plant is a prerequisite for enhancing efficacy, reducing toxicity, or changing the therapeutic range of plant[6]. Heat treatment causes enzymatic processes to occur which lead to significant changes in phytochemical[7,8].

The processed tamarind leaves very likely have dissimilar antioxidant and phenolic content compare to the fresh or dried leaves. Most researches are focused on chemical and antioxidant properties from dried tamarind leaves, while paying little attention to fresh and stir-fried tamarind leaves. Hence, the aim of this study was to determine the antioxidant and total phenolic content of fresh, oven-dried and stir-fried tamarind leaves as well as the chemical groups. Such study can provide vital information on the effects of preparation methods to the chemical and bioactive components of tamarind leaves that are less well researched.

Materials and Methods

Samples Collection and Preparation

The fresh leaves were collected from Perlis, northern part of Malaysia. The leaves were thoroughly cleaned with distilled water prior to extraction. The cleaning process is important to get rid of unwanted substances. The leaves were separated from their petioles. The fresh leaves were procured and cleaned thoroughly with distilled water while the oven-dried leaves were cleaned and oven dried at 60 °C for 3 hours. Afterward, they were ground using blender and sent for extraction and chemical analysis.

Stir-Fried Leaves

After washing with distilled water, the fresh leaves were air-dried at ambient temperature. The air-dried leaves were stir fried using kitchen stove at medium heat (180 °C) for about 10 minutes until the color turned into brown. Afterwards, the samples were kept in an airtight container prior to extraction and chemical analysis.

Plant Extraction

After the preparation of fresh, oven dried and stir-fried leaves, the leaves were ground into powder forms using a blender. About 0.2 g powders were put into conical flask and 20 mL methanol was added. The mixture was placed in water bath at 40 °C for 3 hours. The sample was filtered using filter paper and the extract was stored in a cap bottle at 4 °C for further analysis.

Determination of total phenolic contents

Total phenolic contents of the extracts were determined using the Folin–Ciocalteu assay developed by[9]. About 0.5 mL of samples was pipetted into 10 mL volumetric flask containing 0.5 mL of Follin-Ciocalteu's reagent then 5 ml of distilled water and 1.5 mL of sodium bicarbonate (Na_2CO_3) solution (w/v=20%) were added. The volume was made up with distilled water. During oxidation of phenolic compounds, phosphormolybdic and phosphortungstic acid, contained in the Folin-Ciocalteu's reagent, were reduced to blue-colored molybdenum and tungsten oxides. After two hours, the absorbance of blue coloration was measured at absorbance 765 nm against a blank sample. The measurements were compared to standard curve of prepared gallic acid solutions (concentration range of 100 to 500 ppm) and expressed as milligram of gallic acid equivalent per 100 g ± S.D. The experiment was done in triplicate.

Determination of Antioxidant Capacity

The 1, 1-diphenyl-2-picryhyradrazyl (DPPH) radical scavenging assay is a typical method to measure the antioxidant capacity of plant extracts and natural compounds. The free radical scavenging activity of methanolic leaf extract was measured using the method described by[10]. About 2 mL of methanolic DPPH solution was added to 200 µL of water extract of leaves and then was added with methanol to make final volume of 3 mL. After 60 min, the absorbance was measured against methanol as a blank at 517 nm using spectrophotometer[11]. The free radical scavenging activities of the tested samples were compared with a control. Percentage inhibition of the DPPH radicals was calculated using the formula below:

Percent of inhibition (%) = Ac of control - As of sample / Ac of sample] x 100
Ac = The absorbance of control
As = The absorbance of sample

FTIR Analysis

Perkin Elmer Universal Attenuated Total Reflectance (ATR) sampling accessory was used to obtain the

FTIR spectra of fresh and stir-fried tamarind leaves. The samples of leaves were in the form of powder. Data collection and processing were tested by Spectrum version 6.2.0.0055 software. The FTIR spectrum was used to distinguish the functional groups of the active components found in plant sample based on the peaks values in the region of IR radiation.

Statistical Analysis

All the experiments were measured in triplicate and results were expressed as means ± S.E. Student's Analysis of variance ANOVA (Microsoft Excel 2010 Workbook) was performed to analyze for statistically significant results. Regression analyses (R) were performed to confirm correlation of three data sets (Microsoft Excel 2010 Workbook).

Results and Discussion

Total Phenolic Content

Phenolic compounds are major plant secondary metabolites. They present in a large amounts in plant based foods and valuable for human health. Total phenol contents (TPC) of the fresh, oven-dried and stir-fried tamarind leaves were quantified from a calibration curve (y = 0.0053x – 0.487) of gallic acid and expressed as mg/g. The TPC of all samples are summarized in Table 1.

Table 1: Total phenolic content and % DPPH radical-scavenging inhibition of fresh, oven-dried and stir-fried tamarind leaves

Treatments	Total phenolic content (mg/g)	% DPPH radical-scavenging inhibition
Fresh	39.308^{a} ± 1.34	16.458^{a} ± 1.53
Oven drying	47.736^{b} ± 1.78	39.028^{b} ± 0.25
Stir frying	139.874^{c} ± 2.22	69.923^{c} ± 0.11

Means within column with the letters a, b and c indicate a significant difference among the treatments by ANOVA at $p<0.05$.

Total phenolic content of tamarind leaves range from 39.31 mg/g to 139.87 mg/g in the three preparation methods. The TPC of tamarind leaves fell within the range of TPC in tamarind reported by[1] and[12]. The stir-fried leaves had significantly highest TPC (139.87 mg/g) while the fresh leaves had the lowest TPC (39.31 mg/g). High TPC of stir-fried tamarind leaves was similar to the TPC of dried spices obtained by[13]. The TPC content of stir-fried tamarind leaves was higher than the TPC of herb infusion[14,15]. This suggests the consumption of stir-fried tamarind leaves extract can provide phenolic content to promote health. Our results show the increase total phenolic contents in tamarind leaves when heat was applied to the samples. These findings are inconsistent with work of [16,17], who found that loss of phenolic content in vegetable and fruit after thermal drying process. However, an increase in the phenolic content in tomatoes dried at 80° for 7 hours has been reported[18]. It appears that 10 minutes heating of stir-frying increases the TPC in the tamarind leaves samples. The results agree with the findings by[19] who reported that short heating time might stimulate the activation of enzyme in the leaves, hence, led to significant increases in the phenolic concentrations of tamarind leaves. Total phenolic content of oven-dried tamarind leaves (47.74 mg/g) is consistent with the TPC of tamarind leaves reported in the literature[1]. Long heating time during oven drying could have degraded some heat labile bioactive substances.

Antioxidant Analysis

Aging and different chronic diseases including diabetes, cancer and cardiovascular diseases could be caused by oxidative stress. Oxidative stress can arise from the excessive formation of free radicals. Antioxidant constituents of the plant material act as radical scavengers, and help in converting the radicals to less reactive species. The three treatments with the highest percentage DPPH radical-scavenging inhibition were in the order: stir-frying (69.92 ± 0.11) > Oven-dried (39.03 ± 0.25) > Fresh (16.46 ± 1.53) (Table 1). Radical-scavenging activities of stir frying treatment

was the highest, suggesting the stir-frying process could have increased amount of phytochemical in short thermal process[20]. Percentage DPPH radical-scavenging inhibition of stir-fried leaves was similar to stir-fried ginger reported by[21]. These indicate short thermal duration increasing the antioxidant activity in sample and decreasing the formation of pro-oxidant. Furthermore, a correlation was observed when comparing the data reported in Table 1 for TPC with DPPH radical-scavenging inhibition (r^2 = 0.877). The antioxidant capacities were mainly attributed to the total phenolic content in the tamarind leaves extracts.

FTIR Analysis

The FTIR spectra of fresh, oven-dried and stir-fried tamarind leaves are shown in Figure 1. An increased intensity of the aldehydes group (2918.81-2918.75 cm^{-1}) observed in the spectrum of oven-dried and stir-fried tamarind leaves suggests the heating effects of oven-drying and stir-frying provoke the formation of aldehydes. The presence of aldehydes compounds of the food after thermal process was also reported by[22]. The FTIR analysis of fresh tamarind leave revealed that the presence of primary alcohol (3367.43 cm^{-1}), amide (1641.92 cm^{-1}) are responsible for the synthesis of nanoparticles[23]. The tamarind leaves treated with oven drying and stir-frying have similar chemical groups which were: NH-stretch (3427.31 cm^{-1}), alkanes (2918.75-2850.18 cm^{-1}), aldehyde (1735.87-1735.10 cm^{-1}), NH bend (1629.85-1629.57 cm^{-1}) and CN-stretch (1264.25-1263.35 cm^{-1}). The amine groups were present in the leaves after stir-frying and oven drying process, indicating that the antioxidant potency of tamarind leaves after heat treatment. In addition, amine groups are known to be radical scavengers quenching singlet oxygen[24]. The intensity of the bands of amine groups for the oven-dried and stir-fried leaves was much higher than that of the fresh leaves, suggesting a reason for the higher percentage DPPH radical-scavenging inhibition for the oven-dried and stir-fried leaves than for the fresh leaves (Table 1).

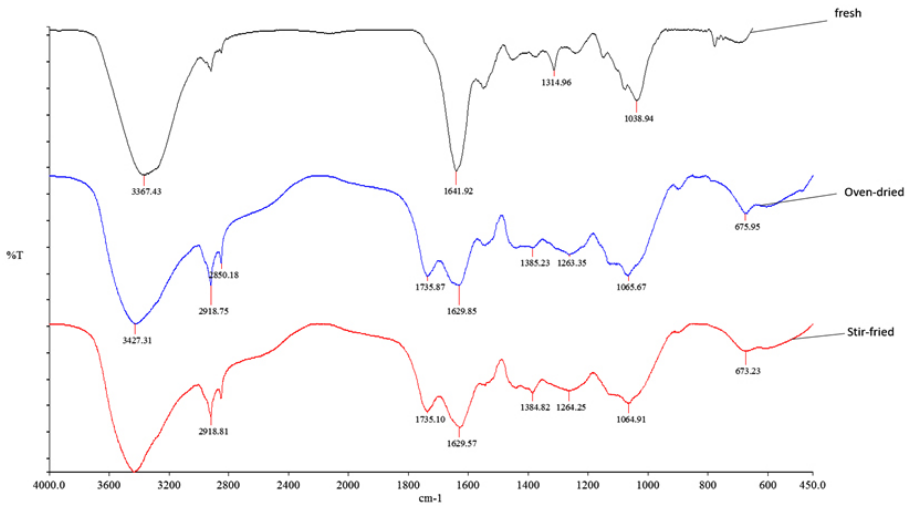

Fig. 1: FTIR spectra of fresh, oven-dried and stir fried tamarind leaves

Conclusions

Total phenolic contents and percentage DPPH radical-scavenging inhibition of stir-fried tamarind leaves were the highest than those of oven-dried and fresh leaves. The FTIR spectral data suggest that the heat treatment increased the amine groups as well as the antioxidant capacity of the tamarind leaves. To increase the antioxidant capacity and total phenolic content, the tamarind leaves should be prepared in a stir-frying treatment. Further studies are underway to determine the nutrient compositions of fresh, oven-dried and stir-fried tamarind leaves.

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