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Effect of Processing Temperature on Antioxidant Activity of *Ficus carica* Leaves Extract

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Abstract: Ethanol is acknowledged as a good solvent for polyphenol extraction and is safe for human consumption. Therefore, it usually used in the extraction process of plant sample. However ethanol should be evaporated to obtain pure extract of the samples. Nevertheless, temperature involved during evaporation process would affect the antioxidant activity of the plant extract. Thus, the purpose of this study was to find out the effect of temperature used during evaporation of ethanol towards antioxidant activity of *Ficus carica* leaves extract. Dried powder of *F. carica* leaves were subjected to three sets of processing method which involve different temperatures: Set A involved maceration with 70% ethanol, followed by evaporation of ethanol in rotary evaporator at 60ºC for 12 hours, set B involved maceration with 70% ethanol, followed by evaporation of ethanol in vacuum oven at 45ºC for 12 hours, while set C involved maceration with 70% ethanol, followed by evaporation of ethanol at 25ºC (room temperature) in fume chamber for 72 hours. Antioxidant activity of extracts from each set were measured using two different *in-vitro* assays, including scavenging abilities on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and total phenolic content (TPC). *F. carica* extract from set B showed the highest antioxidant power in DPPH (3.78 mg/ml) and TPC (266.96 mg GAE/g) compared to set A and C. Thus, it can be concluded that temperature of 45ºC could promote the antioxidant activities, while high temperature would decrease the antioxidant abilities of *F. carica* leaves extract.

Keyword: Antioxidant; ethanol; extraction; *Ficus carica*; temperature.

1. Introduction

Ficus, the fig genus is one of the largest genera of angiosperm which consists of over 800 species of trees, epiphytes and shrubs worldwide. There are many types of species commonly found such as *F. carica*, *F. religiosa L.*, *F. elastic Roxb,ex Hornem* (the rubber tree), *F. benghalensis* (the bayan tree), *F. racemose L.*, *F. deltoidea Jack* and others [1]. *F. carica* is the most common fig plant among other species. Its leaves have been known for centuries of their medicinal benefits as remedy for metabolic, cardiovascular, respiratory, antispasmodic, anti-diabetic and anti-inflammatory treatment [2]. This is mainly attributed to the fact that *F. carica* leaves contain high phenolic compounds with antioxidant properties that are able to prevent health disorders related to oxidative stress [3].

Numerous literatures reported that dietary antioxidant protect against free radicals in the human body [1,4]. It helps to slow down or prevent oxidation by eliminating free radical intermediates and preventing different reactions by being oxidized themselves. Lots of interest to the application of natural antioxidants in medical and food industry has been noticed due to the discovery of these bioactive compounds in food with possible antioxidant activity [5]. Solvent-extraction procedure regularly performed as a recovery of antioxidant components. However nowadays, a clear data on the extraction methodology and requirement are available although it contradicts sometimes depending on the raw materials being used [6]. According to Spigno *et al.* (2007) [6], factors such as types of solvent, extraction methods, processing temperature and duration were parameters to be optimized in order to generate the highest yield of extract and of the maximum extraction quality in terms of the target compounds and antioxidant power.

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Alcoholic solvents have been frequently performed to extract phenolic compounds from natural sources and was shown to give high yield of total extract [7]. Not only that, according to Pinelo *et al.,* (2005), mixture of water and alcohol is more efficient to extract phenolic compounds compared to mono-component solvent system[8]. Besides, there was research focusing on the temperature that would affect antioxidant activity as well. According to previous study, increment in the temperature enhances antioxidant activity but beyond a certain temperature, the antioxidants could be denatured [6]. Since *F. carica* is popular as one of the medicinal plants with its high antioxidant activity, it is important to find out the effect of temperature used during evaporation process of ethanol towards antioxidant activity of *F. carica* leaves extract before it can be used effectively into nutraceuticals, pharmaceuticals or food ingredients in the future.

2. Materials and Method

Materials. *F. carica* dried leaves, 70% and 100% ethanol solution, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) solution, 1M Na_2CO_3, Folin-Ciocalteau reagent, gallic acid, weighing machine, blender, shaker, Whatman No.1 filter paper, hot plate, rotary evaporator RE301 branded BUCHI Malaysia, R-215 with vacuum pump V700 and vacuum controller V850, vacuum oven, fume chamber and spectrophotometer brand Shimadzu UV-1208, Japan.

Sample preparation. The mature sun-dried leaves of *F. carica* were purchased from a local fig farm, Formenniaga which was located at 12, Jalan BPP 5/4, Pusat Bandar Putra Permai 43300 Seri Kembangan, Selangor. The dried leaves were ground into powder form using a blender and kept in a sealed container until it was being used.

Preparation of extract. The dried powdered leaves were divided into three sets (set A, B and C) to undergo different processing temperature (Table 1). The three sets of dried *F. carica* leaves underwent the same type of extraction and different heating process to find out the effect of processing temperature on the antioxidant yield of *F. carica* pure extract.

Table 1 Processing temperature involved in set A, B, C

Set	Process involved
A	Maceration with 70% ethanol – Heated at 60°C in rotary evaporator for 12 hours
B	Maceration with 70% ethanol – Heated in vacuum at 45°C for 12 hours
C	Maceration with 70% ethanol – Evaporated in fume chamber at 25°C (room temperature) for 72 hours

Extraction. The dried powdered leaves were extracted with the same method which was maceration with ethanol. In maceration extraction method, 165 g of dried powdered *F. carica* leaves were soaked with 70% ethanol (1:40, w/v) at 27°C for 72 hours [9]. It was agitated on a shaker with the speed of 70 rpm [10]. 70 % of ethanol was being used as it has contributed to the highest scavenging activity on DPPH radicals [11]. The extract was filtered with Whatman No. 1 filter paper and the marc was remacerated with the current solvent until it was exhausted [9]. Ethanol was fully evaporated once the volume has reduced to 30 % from the initial volume. The liquid extract was kept in refrigerator at 4^0C under it was being used.

Heating. The liquid extract of set A was filled into rotary evaporator RE301 branded BUCHI Malaysia, R-215 with vacuum pump V700 and vacuum controller V850 at 60°C, under reduced pressure of 175 mbar with rotation at the speed of 130 rpm to remove the ethanol completely [12]. While, the liquid extract of set B was poured into 150 ml beakers and placed into vacuum oven at 45°C. The purpose of heating procedure was to evaporate completely ethanol content in the extract so that pure extract could be obtained. However, the liquid extract of set C was poured in beakers and placed in fume chamber at room temperature (25°C) for 72 hours without any heating process.

Antioxidant assays. Two distinct antioxidant assays, which were 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and total phenolic content (TPC) were employed to determine the antioxidant activity as well as total phenolic content in set A, B and C of *F. carica* extract.

DPPH assay. Determination of free radical scavenging activity of the sample was carried out using the DPPH assay which was adopted from Mensor *et al.,* (2001) [13]. DPPH solution was prepared by adding 5.9 mg of DPPH powder into 100ml of 100% ethanol and mixed well together. Then, the sample was diluted with 100% ethanol to final concentration of 20, 10, 5 and 2.5 mg/ml respectively. 3 ml of 5.9 mg/100 ml DPPH solution was added to 50 ul of each sample and incubated in the absence of light for 15 minutes before the absorbance was taken using spectrophotometer at 517 nm. The DPPH blank is used as control. The radical scavenging activity (Equation 1) and EC_{50} were calculated. The experiment was done in triplicate.

Radical Scavengers Activity (%)

$$= \frac{\text{Abs DPPH-Abs sample}}{\text{Abs DPPH}} \text{ x } 100\% \qquad (1)$$

Where:
Abs sample = absorbance of sample in ethanolic DPPH solution
Abs DPPH = absorbance of DPPH solution.

TPC assay. The method used to determine the total phenolic content was adapted from Konyalıoğlu *et al.* (2005) [14]. 100 µl of sample was added into 2.0 ml of 1M Na_2CO_3. 100 µl of Folin-Ciocalteau reagent (1:1, v/v) was mixed and the solution was let to stand at room temperature within 30 minutes. The absorbance reading was taken at 750 nm in triplicate. Control sample was prepared using the same method without test compound or standard. Gallic acid was use as the standard and prepared in concentrations of 0, 20, 40, 60, 80 and 100 µg/ml. This was because gallic acid was commonly used as a reference compound. The determination of phenolic concentration is done by comparing the sample with the standard calibration curve of gallic acid. Total phenolic content value was expressed as gallic acid equivalents (mg/g).

Statistical analysis. The statistical analyses were performed by a one-way ANOVA using minitab. All the results were expressed as means ± SD. to show variations in the various experimental. Differences are considered significant when $p < 0.05$ [15].

3. Results and Discussions

DPPH. Table 2 shows the results of DPPH assay. From the result, set B showed the highest radical scavenges capability compared to set A and C extracts due to the temperature the extracts were subjected to in order to evaporate the ethanol (45ºC for set B, 60ºC for set A and 25 ºC for set C). This was explained by Yi & Wetzstein (2011) [16], and supported by Pinelo *et al.,* (2005) [8] whose found out that temperature more than 50ºC resulted in thermal degradation of phenolic compounds which leaded to decrease in antioxidant activity. This was because phenolic compounds were simply oxidized and hydrolyzed at high temperature.

However, there was also research showing that a slight increase of extraction temperature could actually contribute to higher analyte solubility by maximizing both mass transfer rate and solubility. Furthermore, the surface tension and viscosity of the ethanol solvent were reduced at elevated temperature, thus helped the solvents to improve the extraction rate by reaching the sample matrices [17]. This was able to justify the reason set B *F. carica* extract underwent heating process in vacuum oven (40ºC) but still retained the highest radical scavenging ability.

On the other hand, there were quite huge difference between set B and set C although set C was not going through any type of heating. This is because according to Jeong *et al.,* (2004) [18], antioxidant activity was significantly affected by heating temperature and duration of treatment. Ethanol was chosen to be used in the extraction method as it exhibit the highest efficacy, generating greatest number of phenolic compounds, compared to other solvents.

Table 2 Antioxidant activity (EC_{50}) of *F. carica* with different extraction temperatures in DPPH radical scavenging assay

Set	EC_{50} (mg/ml)
A	20.55 ± 0.86^{c}
B	3.78 ± 0.19^{a}
C	16.32 ± 0.51^{b}

TPC. TPC results showed that total phenolic content of *F. carica* extract with different processing methods were arranged in the order of Set B (266.96 mg GAE/g) > Set A (119.11 mg GAE/g) > Set C (46.99 mg GAE/g)

(Table 3). It could be seen that 70% ethanolic maceration methods at higher temperature in set A and B contained higher phenolic content compared to set C at room temperature. The results obtained corresponded to the literature review studied on efficiency of 70% ethanol as the solvent that always results in the maximmum extraction yield with highest presence of phytoconstituents such as saponins, carbohydrates, tannins, alkaloids and flavonoids in comparison to other solvents including water, chloroform and petroleum ether [19]. Besides, 3-O- and 5-O-caffeoylquinic acids, bergapten, quercetin 3-O-glucoside, quercetin 3-O-rutinoside, psoralen, ferulic acid and organic acids such as citric, oxalic, quinic, malic, fumaric and shikimic acids which were reportedly found abundance in *F. carica* leaves stated in literature review were more soluble in ethanol than in water, leading to higher phenolic content [20].

Moreover, it is believed that set B of *F. carica* extract contained higher phenolic compounds compared to set A and C extract due to the temperature the extracts were subjected to in order to evaporate the ethanol (45ºC for set B, 60ºC for set B and 25ºC for set C). Most polyphenols and flavonoids were heat sensitive compounds although extraction in high temperature normally promotes mass transfer process, which led to faster extraction rate [21]. The research also indicates that set C has lesser phenolic content because it has no inclination of temperature to enhance the solubilility of solute. Pinelo. *et al.*, (2005) [8] agreed that rising the temperature helps extraction by enhancing the diffusion coefficient and solubility of solute which resulted in higher total phenolic content in the extract. However, the phenolic compounds' stability was affected and denaturation of the membranes occured at temperatures more than 50ºC despite the positive increase in phenolic content due to a higher temperature used.

Table 3 Total phenolic content of *F. carica* samples with different processing temperatures

Set	Total phenolic content (mg)
A	119.95 ± 1.17^{a}
B	266.96 ± 1.85^{b}
C	52.21 ± 0.75^{c}

4. Conclusions

In conclusion, temperature used during processing highly affected the antioxidant activity of *F. carica* leaves. Results showed that maceration with 70% ethanolic extract, followed by heated in vacuum at 45ºC for 12 hours had the highest antioxidant power in DPPH assay. From this study, 45ºC was found to be the best processing temperature for *F. carica* leaves extract to retain more antioxidant properties compared to maceration with 70% ethanolic extract followed by heated in rotary evaporator at 60ºC for 12 hours and maceration with 70% ethanolic extract followed by evaporation at 25ºC for 72 hours in room temperature. It could be stated that temperature more than 50ºC resulted in thermal degradation of phenolic compounds in the leaves and upon any inclination of temperature would not release as much as antioxidant content compared to the evaporation at 45ºC.

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