

## Original Article

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### Evaluation of the Effect of Temperature on Total Phenolic Content, Total Flavonoid Content and Free radical Scavenging Activity in Chia Seeds (*Salvia hispanica* L.) Tea

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#### Abstract

Chia seeds are obtained from the plant *Salvia hispanica* (Family: Lamiaceae) and they are known to have multiple health benefits. This study was aimed to provide preliminary data on the optimum temperature in Chia seed tea preparation. The main objective of this study was to examine on the effect of preparation temperatures on the *in vitro* antioxidant activities of Chia seed tea. The Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and radical scavenging activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of Chia seeds were determined by using colourimetric methods. Tests were performed in triplicate and results were analysed using Tukey's test. Based on the result obtained, a significantly increased ( $p < 0.05$ ) in the TPC was observed in the Chia seed tea at 80°C (4.3%) when compared to 4°C. Moreover, a significant increase ( $p < 0.05$ ) in the TFC was also observed in Chia seed tea at 80°C (69.7%) when compared to 4°C. Additionally, Chia seed tea at 80°C shows a significant increase ( $p < 0.05$ ) of 13.3% when compared to 4°C in radical scavenging activity. In conclusion, 80°C is the optimum temperature for preparation of Chia seed tea as it shows the highest TFC and hence the highest free radical scavenging activity was observed.

**Keywords:** *Salvia hispanica*, Antioxidant, Total Phenolic Content, Total Flavonoid Content

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## Introduction

Chia seeds are tiny black seeds from the plant *Salvia hispanica* belongs to the family of Lamiaceae which is related to the mint. Chia seeds were an important food for the Aztecs and Mayans back in the day. They prized them for their ability to provide sustainable energy. In fact, “chia” is the ancient Mayan word for “strength”. Despite their ancient history as a dietary staple, Chia seeds became recognized as a modern-day superfood only recently. In the past years, they have exploded in the popularity and are now consumed by health-conscious people all over the world because they are packed with abundance of nutrients and are particularly known for their high antioxidant content. <sup>[1]</sup> These antioxidants protect the sensitive fats in the seeds from going rancid. Antioxidants fight the production of free radicals, which can damage cell molecules and contribute to aging and diseases like cancer. <sup>[2]</sup> Some examples are Vitamin C and E,  $\beta$ -carotene, selenium, manganese, as well as flavonoids, phenol, polyphenols, and phytoestrogens. <sup>[3]</sup> However, the method of consumption varies all over the world, and antioxidant activity varies with the temperature of the preparations.

Nutritionally, chia seeds are one of the most-concentrated sources of alpha-linolenic acid (ALA), a plant-based omega-3 fatty acid. They are also high in dietary fibre, protein, calcium, iron, magnesium, zinc, and antioxidants. <sup>[4]</sup> Chia seeds are easily digested, so they can be eaten in whole. Extensive research on phytochemical of Chia seeds have been done in recent years, and various active ingredient including essential food fatty acids and phenolic compounds have been reported as well. Chia contains the highest amount of these fatty acids out of all known food source, which contains about 64%  $\omega$ -3 and 19%  $\omega$ -6 fatty acids. <sup>[5]</sup> Dry chia seed on the other hand, contains 8.8% of phenolic compound, and this was correlated with the presence of high level of caffeic acid, chlorogenic acid and quercetin. <sup>[4]</sup> In another study, the phenolic compounds were recorded as 2,639g of gallic acid equivalent/kg of dry sample. The flavonoid content was 0.162g equivalent of quercetin/kg dry sample. <sup>[6]</sup>

The seeds from this plant are boiled in water to from decoction for anti-hyperlipidemia and anti-hypercholesterolemia, anti-diabetic, diuretic and laxative purposes. <sup>[7]</sup> The crude extract of Chia seeds was reported to contain tannins, saponins, flavonoids, alkaloids, proteins, cardio glycosides, and phenols. <sup>[8]</sup> There are increasing citizens in Malaysia that are convinced in taking chia seeds as part of their everyday-diet due to its nutritional values. People are taught to mixed chia seeds in their drinking water as well as sprinkled over their favourite dishes. Chia seed remains at top of the list due to convenience in consuming (unlike flaxseeds which are best consumed in ground form), it does not affect salt intake (as pumpkin seeds are best consumed in roasted form which is usually salt-roasted) and it does not relatively cause allergies (an estimated value of 0.2% of the population in areas where sesame seeds are commonly used, are allergy to sesame seeds). <sup>[9]</sup> Temperature is one of the most important factors that will affect antioxidant activity. A decrease in activity of present or added antioxidant can be seen when heating

causes an acceleration of initiation reactions. Up to our best knowledge, there is no study reported on the effect of preparation temperature on the antioxidant activity in Chia seed tea. The objectives of this study were to determine the antioxidant level in Chia seed when added to water at different temperature and to identify the optimum temperature for Chia seed tea preparation with highest antioxidant level.

## Methods

### Materials

Chemicals used were aluminum chloride, ascorbic acids, catechin, Folin-Ciocalteu reagent, gallic acids, potassium acetate, sodium carbonate and DPPH solution. All chemicals used were of standard analytical purity grade.

### Chia seed tea Preparation

Chia seeds were purchased from a hypermarket in Kuala Lumpur, Malaysia and was identified by a botanist. A total of 0.4 g of Chia seeds were weighed and added into 40 mL of distilled water at 4°C, 25°C, 60°C, and 80°C. Mixtures were incubated for 15 minutes.

### Phytochemical Screening

#### *Determination of total phenolic content (TPC)*

The TPC of Chia seed tea extract was measured with Folin-Ciocalteu method described by Kit *et al* (2016) with some modification. <sup>[10]</sup> Briefly, 0.1 mL of Chia seed tea was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 0.25 mL of 2% (W/V) sodium carbonate. The final mixture was left for 30 minutes in the dark before reading at 750 nm with spectrophotometer. All measurements were conducted in triplicates and the data were expressed as mg Gallic acid equivalent per g dry weight of extract based on Gallic acid calibration curve. This experiment was repeated in triplicate (n=3).

#### *Determination of total flavonoids content (TFC)*

Equal volume of 10% (W/V) Aluminum chloride and 0.1 mL of 1 M Potassium acetate (1 M) were added to 0.5 mL of Chia seed tea, followed by 2.8 mL of distilled water. The solutions were mixed and incubated at room temperature for 30 minutes before the absorbance was taken at 435 nm. <sup>[11]</sup> The flavonoid concentration was expressed as mg catechin per g of extract. This experiment was repeated in triplicate (n=3).

### Determination of *In vitro* Antioxidant Activity

#### *Determination of radical scavenging activity by DPPH Assay*

Free radical scavenging activity of Chia seed tea was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay based on the method described by Sharma *et al* (2009) with slight modification. <sup>[12]</sup> Ascorbic acid

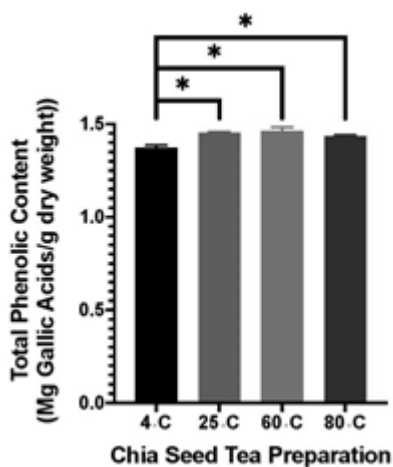
(20 mg/mL) was used as positive control in this study. A volume of 1 mL of DPPH solution (1 mM) was mixed with 0.5 mL of 1% (W/V) Chia seed tea extract. All samples were mixed using vortex stirrer and incubated in dark for 40 minutes at room temperature. Absorbance was recorded using spectrophotometer at 517 nm. This experiment was repeated in triplicate (n=3).

### Data Analysis

Results were expressed as mean  $\pm$  standard deviation. The differences between groups were determined by analysis of variance (ANOVA) followed by Tukey's test. Statistical analysis was conducted using GraphPad Prism 8.2.0<sup>®</sup>. A value of  $p < 0.05$  was considered as significant.

### Results

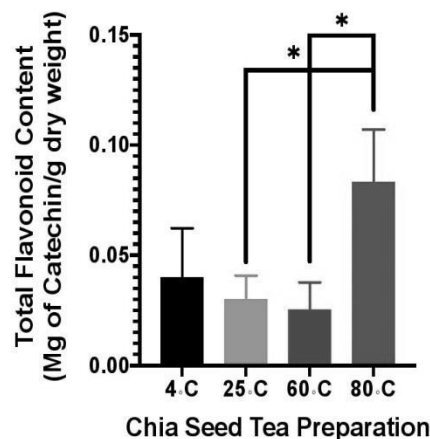
The total phenolic content (TPC), total flavonoid content (TFC) and radical scavenging activity by DPPH in Chia seed tea were shown in the Figure 1, 2 and 3. Based on the results obtained, a significantly increase ( $p < 0.05$ ) in the TPC was observed in the Chia seed tea at 25°C (5.4%), 60°C (6%), and 80°C (4.3%) when compared with 4°C. On the other hand, a significantly increase ( $p < 0.05$ ) of 63.9 % and 69.7 % in TFC was observed in the Chia seed tea at 80°C when compared to 25°C and 60°C respectively. DPPH scavenging activity in Chia seed tea at 80°C ( $p < 0.05$ ) was highest followed by chia seed tea at 60°C, 25°C and 4°C.



**Figure 1. Total Phenolic Content of Chia Seed Tea Prepared at Different Temperature**

n=3; Data = mean  $\pm$  standard deviation (S.D),  
Analysed using Tukey's test

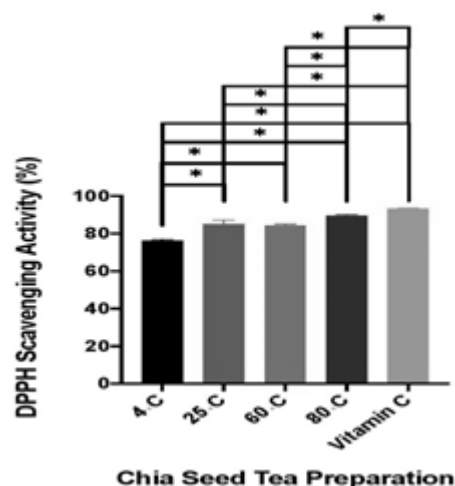
\* $p < 0.05$  significant differences when compared with the other preparations.



**Figure 2. Total Flavonoid Content of Chia Seed Tea Prepared at Different Temperature**

n=3; Data = mean  $\pm$  standard deviation (S.D),  
Analysed using Tukey's test

\* $p < 0.05$  significant difference when compared with other preparations.



**Figure 3. Radical Scavenging Activity by DPPH Assay of Chia Seed Tea Prepared at Different Temperature**

n=3; Data = mean  $\pm$  standard deviation (S.D),  
Analysed using Tukey's test

\* $p < 0.05$  significant difference when compared with other preparations.

for better understanding on the beneficial effects of Chia seeds.

## Discussion

Preparation temperature for Chia seed tea in this study were chosen based on the common way of tea consumption by human at temperature for cold, room temperature, warm, and hot (4°C, 25°C, 60°C, and 80°C). Phenolic compounds and flavonoids were found to be present in all Chia seed tea preparations. Heat treatment has a positive effect on the phenolic content, flavonoid content and radical scavenging activity of Chia seed tea as they are generally increased with increasing temperature from 4°C to 80°C. This suggests that the seeds might contain flavonoids and phenolic compounds which are thermally stable.

Phenolic compounds can be subdivided into phenolic acids, flavonoids, tannins, coumarins, ligans, quinones, stilbens and curcuminoids.<sup>[13]</sup> In a study reported by Piluzza G & Bullitta S (2011), phenolic content could be used as an indicator of antioxidant properties.<sup>[14]</sup> Their antioxidant properties are related to their phenolic rings and hydroxyl group.<sup>[15]</sup>

The presence of flavonoids in Chia seeds may be responsible for the highest scavenging activity observed in Chia seed tea prepared at 80°C. These findings are in line with the study reported by Molaveisi *et al* (2019), when the antioxidant activity in jujube honey increases with temperature.<sup>[16]</sup> Moreover, in a study reported by Hove *et al* (1944),  $\alpha$ -tocopherol activity increased with increasing temperature.<sup>[17]</sup> (A study reported by Van Acker *et al* (2000) states that  $\alpha$ -tocopherol can be replaced by flavonoids as a chain-breaking antioxidant.)<sup>[18]</sup> Therefore, boiling Chia seed at 80°C for 15 minutes was found to be the optimum temperature to prepare the Chia seed tea with highest antioxidant activity and total flavonoid content when compared with other temperature tested in this study.

Higher antioxidant property of Chia seed tea at 80°C is able to scavenge free radical faster. This helps to reduce its capacity to cause cellular damage.<sup>[19]</sup> Its high flavonoids content contributes to the anticancer mechanism by elucidating its point of intervention in cancer cell cycle, including carcinogen inactivation, inhibition of angiogenesis, antiproliferation, cell cycle arrest, induction of apoptosis, antioxidation, and reversal of multidrug resistance.<sup>[20]</sup> Flavonoids also has bactericidal and bacteriostatic effect by damaging cytoplasmic membranes. Additionally, it acts by inhibiting energy metabolism and synthesis of nuclei acids against different microorganisms. With these actions, flavonoids not only have anticancer property but antibacterial and antiviral as well.<sup>[21]</sup>

This study provides preliminary data about the *in vitro* antioxidant activity of Chia seed tea prepared at different temperature. Further studies such as identification of the phytochemicals that responsible for the antioxidant activity and evaluation of the *in vivo* antioxidant activity by using laboratory animals are worthy to be carried out

## Conclusion

In conclusion, the results showed that Chia seed tea at 80°C has the highest antioxidant activity. There was a relationship between the content of flavonoids and free radical scavenging activity *in vitro*, showing that these compounds are the main responsible element for the antioxidant potential of Chia seed tea. Thus, the optimum temperature recommended for the Chia seed tea preparation is 80°C. Hence, preparing the Chia seed tea at 80°C will give the most health benefit to the consumers.

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