Effect of Grinding the Herb and Boiling the Infusion on Total Phenolic Content and Antioxidant Capacity of Herbal Infusions

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Abstract: Herbal infusions are one of the most consumed beverages in the world. The object of this work was to estimate the phenolic content and evaluate the antioxidant capacity of Cumin seeds (*Cuminum cyminum L.*) and Anise seeds (*Pimpinella anisum L.*) infusions, and study the effect of grinding the herb and boiling the infusion on that. The total phenolic content (TPC) was determined by Folin-Ciocalteu method, and the antioxidant capacity was evaluated by two methods, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and FRAP (ferric reducing antioxidant power) assay. The TPC and antioxidant activity have increased by grinding the herb and boiling the infusion. By grinding, the TPC and antioxidant activity have nearly multiplied.

Keywords: Anise, antioxidant activity, Cumin, DPPH assay, Folin-Ciocalteau method, FRAP assay.

INTRODUCTION

Natural antioxidants are more readily acceptable than synthetic antioxidants. Recently, much focus has been given to the involvement of active oxygen and free radicals in aging and in disease processes like heart disease, inflammation, arthritis, immune system impairment and cancer [1].

Most antioxidants isolated from higher plants are polyphenols. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential [2].

Interestingly, many herbs are known to contain large amounts of phenolic antioxidants other than well-known vitamin C, vitamin E, and carotenoids. Phenolic antioxidants in herbs are mainly composed of phenolic acids, flavonoids and catechins [3]. Due to the potential beneficial health effects related to tea drinking, it is interesting to closely determine and compare the chemical composition of different teas and herbal infusions [4].

Cumin is a strong aromatic dried ripe fruit (seed) of *Cuminum cyminum L*. It belongs to the Apiaceae family. Cumin seeds are ancient spices with a strong aromatic smell and warm bitterish taste. Cumin is not only a spice but also has great medicinal value. Cumin is used widely in traditional medicine to treat flatulence, digestive disorders and diarrhea [5].

Anise (*Pimpinella anisum L.*) is an annual herbaceous and a typical aromatic plant belonging to the Apiaceae family [6]. Anise seeds are widely used to prepare herbal teas and also as a flavoring agent [7].

However, there are no reports on how much grinding the herb affects on total phenolic content (TPC) and antioxidant capacity of herbal infusion, and limited reports on how much boiling the infusion before keeping it affects on that, so the object of this work was to investigate the effect of grinding and boiling on the TPC and antioxidant capacity of infusions.

MATERIALS AMD METHODS

Materials

Cumin seeds and Anise seeds were obtained from local markets at Damascus,Syria. Folin-Ciocalteu reagent, gallic acid, Iron II sulfate 7-hydrate, sodium acetate, acetic acid, hydrochloric acid, methanol, 2,4,6 tri (2-pyridyl)-s-striazine (TPTZ) and 1,1Diphenyl-2-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich. sodium carbonate anhydrous, ascorbic acid and Iron III chloride 6-hydrate were obtained from Scharalau chemie.

Infusion Preparation

In order to simulate household brewing conditions for a cup of herbl infusion, the herbal infusions were

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prepared using an aqueous extraction procedure. 1.5 g of herb samples were poured with 150 ml of distilled water heated to 90ºc, and kept for 10 minutes at room temperature, then filtered through whatmann no.4 filter paper.

Determination of Total Phenolic Content

Total phenolic contents were determined using Folin-ciocalteu reagent according to Slinkard and Singleton method (1977) [8], using gallic acid as a standard phenolic compound, with slight modifications. Briefly, diluted sample (250 µl) was placed in a cuvette and 1000 µl of 1:10 diluted Folin-ciocalteu reagent were added to the mixture. After 5 minutes, 1000 µl of sodium carbonate 7% solution was added. After 40 minutes of incubation at room temperature, the absorbance of the reaction mixture was measured at 765 nm. Gallic acid was used as a reference standard and the results were expressed as mg gallic acid equivalent (mg GAE/cup). All the experiments were performed in triplicate, and the results were expressed as mean.

$DPPH^{\cdot}$ Assay

Free radical scavenging activity were measured by using 2,2-diphenyl-1-picrylhydrazil ($DPPH^{\cdot}$) according to a modified method by Wu *et al*. (2006) [9]. Aliquot (50 µl) of the tested sample was placed in a cuvette, and 2 ml of $6x10^{-5}$ M methanolic solution of $DPPH^{\cdot}$ were added. The decrease in absorbance at 517 nm was determined after 15 minutes for all samples. Methanol used as blank. The absorbance of the $DPPH^{\cdot}$ without antioxidant was measured daily (control). All determinations were performed in triplicate.

$$\% \text{ inhibition} = A_C - A_A / A_C \times 100$$

A_C: the absorbance of the control, A_A: the absorbance with antioxidant at 15 min.

Ferric-Reducing Antioxidant Power Assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie and Strain (1996) [10], with slight modifications. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe(II)-Tripyridyltriazine compound from colorless oxidized Fe(III) form by the action of electron donating antioxidants. Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM iron(III) chloride solution in proportion of (10:1:1), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37ºc in a water bath prior to use. 50 µl of sample were placed in a cuvette and 1.5 ml of the FRAP reagent was added. The absorbance of the reaction mixture was then recorded at 593 nm after 5 min. The standard curve was constructed using iron(II) sulfate solution (200- 1400 µM) and the results were expressed as µM Fe. All the measurements were carried out in triplicate and the mean values were calculated.

RESULTS AND DISCUSSION

Effect of Grinding the Herb on TPC and Antioxidant Activity

The Folin-Ciocalteu method was used to measure the TPC of Cumin and Anise infusions. It relies on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline media. It is simple, reproducible and used in many studies. The antioxidant capacity of the plant extracts depends on not only the composition of the extract, but also on the test system. Therefore, it is essential to perform more than one type of antioxidant capacity measurement of antioxidant action. The antioxidant capacity was evaluated by two methods, DPPH and FRAP assays [11].

Table **1** shows the TPC and antioxidant activity of Cumin and Anise infusions, using the seed form and the ground form. As shown in Table **1**, infusions that prepared using the ground form of both, Cumin and Anise, had the highest TPC and antioxidant capacities based on a combinative consideration of the results obtained by FRAP and DPPH assays, and the values have nearly multiplied. Increasing surface area as a result of grinding enhances and accelerates the extraction rate, due to increased surface ratio and improved diffusion of compounds from solid materials. Cumin infusion exhibited higher values for TPC, DPPH and FRAP compared to Anise infusion.

Effect of Boiling the Infusion before Keeping it at Room Temperature

Table **2** shows the TPC and Antioxidant activity of Cumin and Anise infusions, without and with boiling the infusion for 1 minute before keeping it for 10 minutes at room temperature. As shown in Table **2**, boiling for a very short time (1 min) had a significant increase on the TPC and antioxidant activity (DPPH and FRAP). The TPC and antioxidant capacity of the infusion increased as the extraction temperature increased, and this suggested that Cumin and Anise phenolics are relatively stable under high temperature conditions.

Table 1: TPC Expressed as mg GAE/cup (150 mL) and Antioxidant Activity by DPPH and FRAP Methods of Cumin and Anise Infusions, Using Seed and Ground Forms

	Form	TPC[a]	DPPH[b]	FRAP[c]
Cumin	seed	13.59 ± 0.09	31.06 ± 1.00	98.58 ± 2.9
	ground	26.99 ± 0.29	61.38 ± 1.65	174.88 ± 6.4
Anise	seed	11.08± 0.61	27.60 ± 0.41	73.82 ± 0.79
	ground	25.99 ± 0.97	47.28 ± 1.7	156.66 ±1.12

Values expressed as mean of three replicate ± standard deviation.
DPPH = (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.
FRAP = Ferric reducing antioxidant power assay.
TPC = Total Phenolic Content.
[a]mgGAE/cup (150 mL), [b]% inhibition, [c]µM Fe/cup (150 MI).

Table 2: TPC Expressed as mg GAE/cup (150 mL) and Antioxidant Activity by DPPH and FRAP Methods of Cumin and Anise Seeds Infusions, without and with Boiling the Infusion for 1 Minute before Keeping it for 10 Minutes at Room Temperature

	Boiling for 1 min	TPC[a]	DPPH[b]	FRAP[c]
Cumin	without	13.59 ± 0.09	31.06 ± 1.00	98.58 ± 2.9
	with	14.24 ±0.08	36.35 ± 0.66	109.16 ±0.24
Anise	without	11.08± 0.61	27.60 ± 0.41	73.82 ± 0.79
	with	11.45 ± 0.12	33.89 ± 0.11	97.41 ± 3.00

Values expressed as mean of three replicate ± standard deviation.
DPPH = (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.
FRAP = Ferric reducing antioxidant power assay.
TPC = Total Phenolic Content.
[a]mgGAE/cup (150 mL), [b]% inhibition, [c]µM Fe/cup (150 ml).

Correlation between the TPC and DPPH Method

Simple linear regression was used to analyse the correlation between the TPC and DPPH values. Figure **1** shows a significant correlation (R^2= 0.872) between TPC values and DPPH values, which implied that the phenolic compound could be the main contributor of the radical scavenging activity of these infusions. The result was in agreement with many previous studies (Battaieb *et al*. 2010 [12], Roby *et al*. 2013 [13], Horzic *et al*. 2009 [4], Parego *et al*. 2002 [14], Allaghadri *et al*. 2010 [15]).

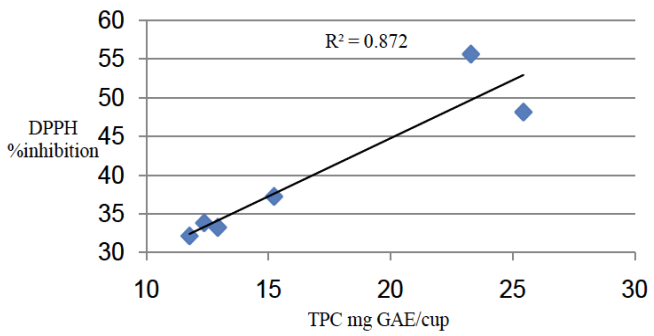

Figure 1: Correlation between total phenolic content (TPC) and DPPH values. DPPH = (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, TPC = Total Phenolic Content.

Correlation between TPC and FRAP Method

To correlate the TPC with the antioxidant capacities obtained from FRAP assay, the correlation coefficient (R^2) was calculated (Figure **2**). R^2 was 0.99, which suggested that the phenolic compounds are the major contributors of reducing oxidant abilities of these infusions. The results were in agreement with many studies (Fu *et al*. 2011 [16], Kazazic *et al*. 2012 [17]), and disagree with (Li *et al*. 2013 [11], Ramkissoon *et al*. 2012 [18]).

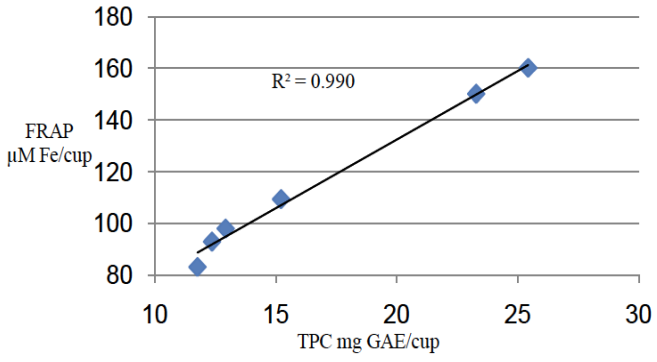

Figure 2: Correlation between total phenolic content (TPC) and FRAP values. TPC = Total Phenolic Content, FRAP = Ferric reducing antioxidant power assay.

Correlation between FRAP and DPPH

As shown in Figure **3**, there was a high correlation (R^2= 0.87) between FRAP and DPPH values, which indicated that the main components contributing to scavenging free radical activities are also responsible for reducing oxidant abilities. The result was in agreement with Kamiloglu 2009 [19].

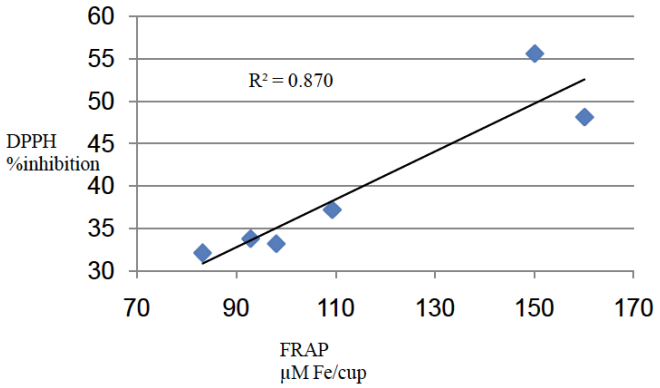

Figure 3: Correlation between FRAP and DPPH values. FRAP = Ferric reducing antioxidant power assay, DPPH= (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

CONCLUSION

The overall evaluation of this study concludes that both Cumin and Anise infusions have good antioxidant potential, especially the ground form. There was also a high correlation between TPC and antioxidant activity, which indicated that phenolic compounds in these infusions could be the main components contributing to scavenging free radical activity and reducing oxidant abilities. This study provides a reliable set of informations regarding TPC and antioxidant capacity of common herbal infusions frequently consumed all over the world, and gives tips for getting the maximum benefit from a cup of herbal infusion. It is recommended to use the ground form of herbs for infusion preparation, and also boiling it for relatively short time before keeping it exhibited a significant increase in antioxidant activity. On the basis of those informations, making herbal infusions a part of our diet may reduce risk of some chronic and age-related diseases.

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