

# Ideal extraction temperature for antioxidants from holy basil and bunching onion

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**Summary.** This study aimed to determine ideal temperature for antioxidants from holy basil and bunching onion. Holy Basil (*Ocimum Tenuiflorum*) and Onion (*Allium Fistulosum*) were extracted with various temperatures ranging from 75 – 100 °C with two solvents i.e. methanol and water (room temperature & boiling). Total phenolic contents (TPC) and total flavonoid contents (TFC) were determined in the extracts by using the Folin-Ciocalteu and Aluminum chloride complex formation assays respectively. Extracts were analyzed in triplicates statistically compared using one-way analysis of variance (ANOVA) and the difference between the mean was ascertained at 95% confidence interval ( $P < 0.05$ ) using Tukey's honest significance test. In both holy basil and bunching onion, the TPC and TFC values for methanolic extracts were significantly ( $P < 0.01$ ,  $P < 0.001$ ) higher than the water extracts. The best temperature among the various temperature used was 85°C where maximum TPC and TFC were observed in the extracts. This study shows that using optimum temperature helps in extraction of maximum antioxidants with methanol.

**Keywords:** culinary herbs, extraction temperature, antioxidants concentration

## Introduction

The interest in the potential uses of natural antioxidants as food preservatives and for health reason has shown an increasing trend (1). Over the past 20 years, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) are being used as food preservatives due to their low cost. However, several researchers have found that there are possible unhealthy effects of synthetic antioxidants and developed countries like Japan, Europe and Canada have banned the use of synthetic antioxidants used in food (2). There are numerous types of natural antioxidants which act as reducing agents to stabilize the free radi-

cals in human body (3) e.g. polyphenols (4). Phytochemicals, (5-7), flavonoids (8), vitamin C (1, 9-12). Apart from health benefits, antioxidants also have many industrial uses, such as preservatives in cosmetics and to prevent the degradation of rubber and gasoline (12). However, synthetic antioxidants used in the food and pharmaceutical industries may be toxic (2) compared to natural sources (13). Holy basil (*Ocimum tenuiflorum* or *Ocimum sanctum*) is an aromatic plant that is native to Eastern World. It is also called tulsi in India and is considered as a holy religious plant among Hindus. Apart from this, it is used as therapeutic ingredients for various purposes (14-16). Furthermore, commonly used as a condiment and garnishing agent in variety of cuisines (17). According to (18), the antioxidants

found in herbs have been identified to have multiple biological effects, including antioxidant activity. The most important chemical constituents in *O. tenuiflorum* extract (mainly in leaf) are eugenol, carvacrol, tannins, methyl eugenol and caryophyllene (19-22). However, other researchers have observed that other essential antioxidants, such as ascorbic acid, carotenoids, tocopherol, tocotrienols, glutathione, phenolic compounds (like flavonoid) and cichoric acid are also present in it (1, 10, 23). Furthermore, *O. tenuiflorum* also has showed to exhibit a hepatic protective effect and can be used in the treatment of hepatic disorders anti-stress, immune modulator, anti-inflammatory, mast cell stabilization, anti-histamine (14) and (24). Onion (*A. fistulosum*) also possesses antioxidants which are characterized by its higher contents of thio-sulfonates (allicin), an antioxidant useful for disease condition (25, 26). Allicin also has anti-bacterial, antiviral, and anti-fungal properties (9, 25, 27, 28). Various studies report that cooking temperature may affect antioxidants concentration/activity (1, 3, 13, 29-33). The optimum release depends on the types and nature of the material and antioxidants (34). Various methods of extraction are used but little attention has been paid to have ideal temperature for extractions (35). Similarly, aqueous and organic solvents would also have positive or negative effect on extraction (36). Therefore, it is would be excellent to have optimal solvent and ideal temperature for extraction of antioxidants (37)

Despite of the wide uses of these two common herbs in cooking, the optimum temperature that would optimize the retention of antioxidant contents of these herbs in cooking is still unknown. Thus, there is a need to study the effect of temperature to identify the suitable temperature that can maximize the retention of the antioxidants in the extract.

## Materials and Methods

This study involved two famous Asian culinary herbs, namely holy basil and bunching onion. Both herbs were purchased from the local market in Kuantan, Pahang, Malaysia. The herbs were then cleaned with distilled water, dried and ground. After grinding, extracted separately with methanol at various temper-

atures (65, 75, 85, 95 and 100°C), water (room temperature) and hot water (65, 75, 85, 95 and 100°C). Two assessments were performed namely Folin-Ciocalteu and Aluminium chloride complex formation for total phenolic contents (TPC) and total flavonoid (TFC) contents. For phenolic contents, a calibration curve of the Gallic acid standards (5, 2.5, 1, 0.5, 0.1 mg/l) was prepared; the absorbance was measured at 760 nm using UV-Vis spectrophotometer and using pure methanol as a blank as described previously by (38). The concentration of phenolic contents in samples was estimated using the formula below:

$$\text{Phenol content mg GAE/g} = [(\text{Slope} \times \text{absorbance}) + c] / \text{Sample concentration}$$

\*c is the y-intercept

Total flavonoid contents were determined by Aluminium chloride complex formation as described by (39). A calibration curve of the quercetin standards (5, 2.5, 1, 0.5, 0.1 mg/l) was prepared and the absorbance was measured at 425 nm using UV-Vis spectrophotometer and using pure methanol as a blank. The concentration of total flavonoid in samples was estimated using the formula below:

$$\text{Flavonoid contents (QE mg/g)} = [(\text{Slope} \times \text{absorbance}) + c] / \text{Sample concentration}$$

\*c is the y-intercept

### Statistical analysis

Statistical analysis of the data was performed by using SPSS (Version 12.1), statistically compared using one-way analysis of variance (ANOVA) and the difference between the mean was ascertained at 95% confidence interval ( $P < 0.05$ ) using Tukey's honest significance test.

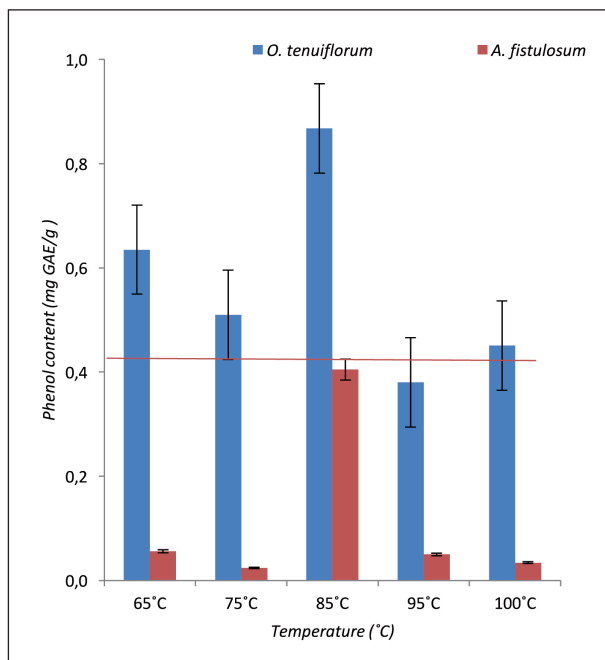
## Results and Discussion

The present study demonstrated some interesting findings for the antioxidant concentration of extracts with different temperatures. As mentioned earlier, the antioxidant concentrations of these two herbs were determined in the form of total phenolic contents (TPC)

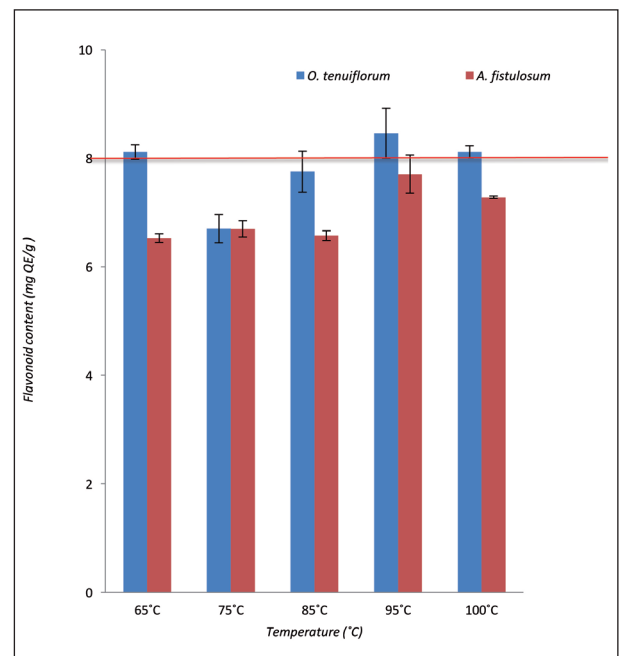
and total flavonoid contents (TFC) and extracted with two different solvent namely methanol and water. The TPC of three different procedure extracts of the two herbs i.e. are presented in the Figure 1 & 2 and Table 1.

Highest ( $P < 0.001$ ) concentration was obtained for the total phenolic contents at 85°C for both herbs compared to the rest of the temperatures used. From these results, it can be deduced that hot water extracts at 85°C exhibits higher content of TPC (Fig. 1). General concept of the effect of the temperature on the antioxidant's concentration is that higher temperature would

lead to lower antioxidant concentration (40). The results of this study reveal that TPC at 85°C was higher than at 65, 75, 95 and 100 °C which might be the suitable temperature. Furthermore, increase in temperature beyond 85 °C would lead to a decrease in TPC yield as it has been observed at 95 and 100°C (Fig 1). Similar, effect has been suggested by (13) that an increase in temperature will lead to an increase in TPC to the maximum concentration whereas there will be a decrease at further increase of temperature and that is what we observed (85 °C). The present study reveals that the effect of tem-



**Figure 1.** Alteration in cooking temperature affects total phenol contents of holy basil and onion. Each bar represents the mean values ± standard deviation, where n = 3.



**Figure 2.** Alteration in cooking temperature affects total flavonoid contents of holy basil and onion. Each bar represents the mean values ± standard deviation, where n = 3.

**Table 1.** The phenolic contents of these extracts were estimated using a standard curve of Gallic acid and expressed as milligrams of Gallic acid equivalents (GAE), the flavonoid contents that were projected using a standard curve of quercetin and expressed as mg of Quercetin equivalents (QE) over weight of sample in gram and

Holy Basil Extracts			Onion Extracts		
Methanol	Water (room Temperature)	Hot Water	Methanol	Water (room Temperature)	Hot Water
Total Phenol Contents (TPC) (mgs GAE/g)					
0.83 ± 0.17 <sup>a</sup>	0.02 ± 0.04 <sup>b</sup>	0.11 ± 0.01 <sup>b</sup>	4.16 ± 0.63 <sup>c</sup>	0.01 ± 0.03 <sup>b</sup>	0.11 ± 0.03 <sup>b</sup>
Total Flavonoid Contents (TFC) (mg of Quercetin equivalents (QE))					
5.04 ± 0.06 <sup>a</sup>	1.57 ± 0.01 <sup>c</sup>	3.77 ± 0.05 <sup>b</sup>	4.91 ± 0.12 <sup>b</sup>	2.14 ± 0.04 <sup>c</sup>	4.81 ± 0.11 <sup>a</sup>

Each value represents the mean ± standard deviation, where n = 3. The vales with different superscripts are significantly ( $P < 0.05$ ,  $P < 0.01$ ) different.

perature on the antioxidant contents of Holy basil and Onion were different from one temperature to another which is due to the thermal effects which varies depending on the type of plants, species (33, 41, 42).

The TFC was higher at 95 and 100°C of hot water extracts as compared to other temperatures for both herbs (Figure 2). Similar result was found by (10) who reports that heating at a higher temperature gave a higher flavonoid content compared to heating at a lower temperature. This has been attributed to the hydroxyl structure of flavonoids that is effectively extracted at 95°C (33). Surprisingly, the results of this study showed that the highest content of TFC was obtained at higher temperature compared to TPC. This was similar to (13) who found that the highest yield of TFC higher temperature (63°C) while the highest yield of TPC were observed at lower temperature (53°C). These authors attribute the results to the differences in the hydroxyl group in the phenolic compounds which responsible for responding differently towards thermal treatment. The specific hydroxyl structure in flavonoids and are acting in a specific different mechanism (43). The variation may be due to the differences in cultivar which may be influenced by genetic factors (41) and varied from one region to another (44).

From Figure 1 Holy basil expressed the highest phenolic contents at temperature of 85°C. As suggested by (44), the total phenolic content which mainly affects the antioxidant activity is a main parameter to determine the overall concentration and activity of antioxidants in the herbs. Therefore, it is considerably valid to conclude that the temperature which resulted in the highest phenolic compounds was the optimum temperature for retention of the antioxidant contents in the tested samples. Figure 1 has revealed that 85°C was the optimum temperature for the optimization of antioxidants concentration in Holy basil.

These results show that both tested herbs possess significant amount of phenolic compounds. The content of TPC was highest in methanol compared to cold and hot water extract similar to the other studies of Holy basil (45) and Onion (44). It can be observed from these results that methanol extracts of both herbs yielded the highest concentration of TFC compared to hot and cold water extracts respectively. This has been reported elsewhere in a study conducted by a group of

(46), who found that the phenolic compounds yield 30% more with methanol compared to the other forms of solvents. The content of holy basil and onion show that both herbs are important for antioxidant activities (10). However, the concentration of the TFC is varied depending on the type of extraction solvent. Similar to the TPC, the highest concentration of TFC was observed in the methanol extracts of Holy basil and Onion. A similar observation has been reported for spices with methanol to be the most effective extraction agent for TFC compared to the other solvents (10).

Phenolic compounds are good sources of antioxidants and have excellent potential in elucidating antioxidants scavenging activity (18, 44, 45). However, the concentration of the phenolic compounds varies depending on the type of extraction (46, 47) and (10). The observed concentration TPC and TFC may have the antioxidant activity and free radical-scavenging capacity in the tested herbs (44, 48, 49) and (44). In fact, the higher the phenolic contents, the higher the free radical-scavenging activity and antioxidant capacity of the sample, and vice versa (49, 50). In the present study, it was found that extraction solvent also influenced the antioxidant contents of these two herbs. Methanol possessed highest yields of total phenols as compared to hot and cold water. Interestingly, the present study revealed that the effect of temperature on the antioxidant contents of both herbs were unique from one temperature to another. Hot water extracts at 85°C had the highest phenolic compounds followed by 65, 95, 100 and 75°C for Holy basil while for Onion; the phenolic compounds yield ranged from highest to lowest was at 85, 65, 75, 95 and 100°C. In fact, the antioxidant activity elucidated by Holy basil of hot water extracts at all manipulated temperatures was considerably higher except for 95°C. Thus, it appears that 85°C was the optimum temperature for the optimization of antioxidants contents for both herbs.

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