

The effect of drying temperature on the antioxidant activity of thyme extracts.

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Abstract

This investigation is aimed at studying the influence of different drying temperatures on the antioxidant activity for thyme leaves. In the present study different spectrophotometric methods (DPPH, FRAP) are using to investigate the antioxidant activities of thyme plants. The results showed that the drying temperature influences the antioxidant activity. The antioxidant activity of all studied extracts decreased with increasing temperature attending decreasing in total phenol. Fresh thyme shows the highest antioxidant activity (0.014 BHTE.mL⁻¹, 0.0064 AAE and 0.0062 GAE).mL⁻¹ extract and total phenol content (8.78 10⁻⁴ mol GAE and 7.50 10⁻³ mol AAE).mL⁻¹ of extract. Whereas thyme dried at 40°C show the lowest antioxidant activity, (0.0042 BHTE, 0.0019 AAE and 0.0018 GAE).mL⁻¹ of plant extract and total phenol contents (4.30 10⁻³ AAE and 4.68 10⁻⁴ GAE).mL⁻¹ of plant extract.

Keywords: Thyme leaves, Drying temperature, Antioxidant, Total phenol.

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Introduction

Phenolic compounds, ubiquitous in plants, are considerable interest and have received more attention in recent years due to their antioxidant and bioactive properties [1-3]. Natural antioxidants in human food increase the resistance toward oxidative damages and they may have essential influence on human health [4]. As a result, there are a lot of advantages consequence the usage of natural antioxidants from food plants. They are safe; the consumers approve them [5] and they have functional properties [6]. Herbs are the most important part of the human food, which have been used as traditional medicine for thousands of years. Thyme is an example of aromatic herb which has been used in food products to supply a flavor exclusive to this plant [7]. Wide thyme in Yemen one of the most important traditional medicine plant. It is a species of flowering plant which belongs to Lamiaceae family [8,9]. Wild thyme is used in food and pharmaceutical preparations [10,11]. In food, it can be used in fresh or dried form, especially for teas [12] and preservative for foods [13]. phenolic acids and flavonoids are the main compounds cause the antioxidant activity of wild thyme [10,12,14-16]. Thymol and carvacrol, have been reported as the main component of thyme [8,17,18]. There are several methods have been developed to determine the antioxidant activity. These methods vary from each other in terms of their principles and experimental conditions. According to the chemical mechanism involved, superior antioxidant activity methods can be divided into two groups: hydrogen atom transfer (HAT) and single electron transfer (ET) mechanisms based methods [19]. Previous researches demonstrate that quality parameters of plants food are influenced by several factors such as drying temperature, oxygen concentration and storage time [20]. Temperature is one of the most important factors affecting on antioxidant activity. Although the initiation of reactions can be accelerated by

heating, the working mechanism of some antioxidants will be changed with variations in temperature led to the decreasing in activity of the antioxidants [21]. There are few studies about the effect of drying temperature on the antioxidant activity of herbal plants. Therefore, the purpose of our study was to investigate the changes in the phytochemical (total phenols) content and antioxidant activity of thyme at different drying temperatures.

Materials and Methods

Plant material

Thyme herbs were collected from the region of Al-shahel, Hajah (Yemen) in the spring, 2015. The leaf of the plants were separated. They were then grinded and stored in the dark, at room temperature until use.

Chemical reagents

All chemicals used were of analytical grade. The TPTZ, DPPH and ferric chloride were obtained from Aldrich. Folin-Ciocalteu phenol reagent, ascorbic acid, anhydrous sodium carbonate, sodium acetate, 98% acetic acid and all metal ions were purchased from BDH.

To prepare antioxidants stock solutions, compounds were dissolved in appropriate solvents: gallic acid and ascorbic acid in deionized water and BHT (butylated hydroxytoluene; 2, 6-di-tert-butyl-4-methylphenol) 0.001 M solution was obtained by dissolving in ethanol. These solutions were prepared daily. Ferric Chloride solution containing 1×10^{-2} M was prepared by dissolving 0.068 g in 25 mL deionized water.

Drying of plant

The samples were labeled and subjected immediately to drying by three different conditions: Without light (shadow) at room temperature, in light for one week at $25 \pm 2^\circ\text{C}$, Under sun light for (1 week) for all of the samples and Dried in oven at different temperature 27, 35, 40°C .

Extraction procedure

In the round bottle flask, 300 g of plant leaves are mixed with 300 mL of water. The mixture was heated under distillation equipment for 4 hours. The organic layer was extracted for three times using diethyl ether. The layer was fired over sodium carbonate anhydrous then the solvent was evaporated. The yellow and intense aromatic volatile oil residue was collected in the dark bottle and kept at 4°C until use. The yield (v/w) of volatile oil was calculated.

Total phenol content (TPC)

This method determines phenols and oxidized substances by producing a blue color from reducing yellow hetero poly phosphor molibdate tungstate anions [22]. In brief, 0.01 mL of each extract ($0.1 \text{ mL} \cdot 1 \text{ mL}^{-1}$) was added to 1 mL of a 1:10 diluted Folin-Ciocalteu reagent. After 5 min, 1 mL of a Na_2CO_3 solution (7.5%w/v) was added. The volume was made up to 3 mL with DW and the reaction mixture was left at room temperature for 30 min in the dark. The absorbance of the solution was read at 750 nm against water blank. The optical density (OD) was compared to a standard curve prepared with 1 to $10 \mu\text{mol} \cdot \text{L}^{-1}$ gallic acid and $8\text{-}80 \mu\text{mol} \cdot \text{L}^{-1}$ ascorbic acid. Results are expressed as gallic acid equivalents (GAE) and ascorbic acid equivalents (AAE). Values ($n=5$) were expressed as means \pm standard deviations.

Determination of Antioxidant Activity

Ferric-reducing power (FRAP) assay

The determination of reducing capacity was performed by mixing the FRAP reagent incubated at 37°C with the samples. The FRAP reagent was prepared from 300 mM acetate buffer, and 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) and ferric chloride hexahydrate (20 mM). These were mixed in the ratio 10:1:1. The samples were measured in triplicate. The absorbance was measured at $\lambda=593 \text{ nm}$. Standard curves was

plotted using different concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, ascorbic acid, gallic acid and BHT. In the FRAP method, the antioxidant activity of the herbs plant tested was calculated. The values obtained were expressed in mol Fe(II) , ascorbic, gallic and BHT. mL^{-1} of plant extract.

DPPH assay

The DPPH assay determined the ability of extracts to scavenge the DPPH radical [22]. A solution of DPPH (30 mM) in pure methanol was prepared. The dilutions were made for the samples (1:10). 40 μL of each extract and ascorbic acid solution were transferred into an eppendorf tube. Each concentration was tested in five plicate. 1 mL of DPPH solution transferred to mixture with sample solution and full up to 2 mL with ethanol. After that the mixture was Shake and left to stand at the room temperature for 30 min. Absorbance was measured at 517 nm, using 2 mL of absolute ethanol as blank. Control solution was prepared by mixing 1 mL ethanol and 1 mL DPPH ($3 \cdot 10^{-4} \text{ M}$) for each experiment. % inhibition was calculated by following equation:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \cdot 100.$$

Statistical analysis

Experimental results are expressed as means \pm SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$). The IC50 values were calculated from linear regression analysis.

Results and Discussion

Analysis of total phenols content

Phenolic compounds in herbs are the most plentiful antioxidants in the human diet. Due to the antioxidant properties of phenolic compounds they are considerable interest [20]. The concentration of total phenols in the extracts was determined, and the results are shown in Table 1 by reference to standard curves (Figure 1).

Initial phenolic compound contents of the fresh thyme extract from the leaves of thyme had the highest phenol content with largest amount of extract oil. The dried thyme in oven at 40°C has the smallest amount of extract oil.

Table 1. Total phenols content for thyme extracts drying at different conditions.

Plant extract	The yield of extracted		Total phenols	
	Extract in mL/300 g herbs	Extract yield v/w%	mol AAE . mL^{-1} of *extract	mol GAE . mL^{-1} of *extract
Fresh thyme	3.5	1.2	$7.50 \times 10^{-3} \pm 0.007$	$8.78 \times 10^{-4} \pm 0.007$
Dried thyme in shadow	3.1	1.03	$6.50 \times 10^{-3} \pm 0.008$	$7.29 \times 10^{-4} \pm 0.006$
Dried thyme under sun light	2.9	0.96	$6.20 \times 10^{-3} \pm 0.008$	$6.80 \times 10^{-4} \pm 0.008$
Dried thyme at 27°C	2.6	0.87	$5.83 \times 10^{-3} \pm 0.008$	$6.46 \times 10^{-4} \pm 0.007$

Dried thyme at 35°C	2.3	0.76	$5.49 \times 10^{-3} \pm 0.006$	$6.12 \times 10^{-4} \pm 0.007$
Dried thyme at 40°C	1.6	0.5	$4.30 \times 10^{-3} \pm 0.008$	$4.68 \times 10^{-4} \pm 0.008$

* Means of three determinations \pm standard deviation

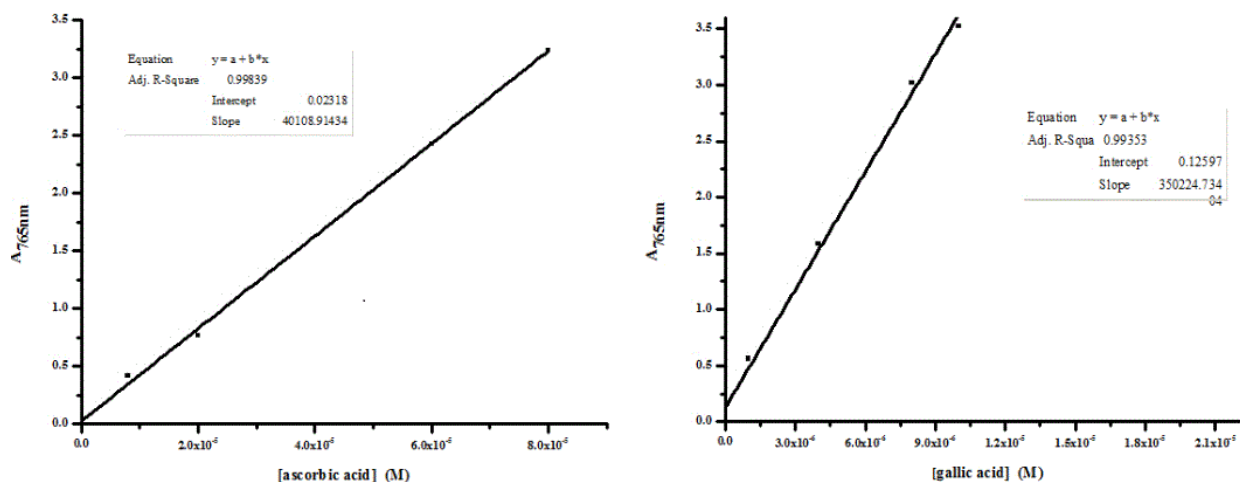


Figure 1. The calibration curves of *a*-ascorbic acid *b*-gallic acid (mol/L) against absorption at 765 nm.

It showed the lowest value of the total phenol content as shown in Table 1. This observation could be explained in term of influence of the heat treatment, which might cause a degradation of these phenolic compounds. This result was in agreement with reports [21].

DPPH scavenger method

This method is based on the measurement of the reducing power of antioxidants towards DPPH+. The ability of antioxidants can be estimated by measuring the decreasing of absorbance [23]. The results of our present investigation showed that the variation of drying temperature affect the antioxidant activity of the thyme oil extracts as presented in the Figure 2. The antioxidant activity of all thyme oil extracts decreased with increasing drying temperature. The activity of free radical scavenging can also be expressed as the concentration which causes the scavenging DPPH (IC50) activity of 50% [24]. In present investigation, the drying temperature can affect the value of the IC50.

Fresh thyme has the highest IC50 at all whereas dried thyme at 40°C has the lowest IC50 values. This result can be explained by the high amount of antioxidant compounds in fresh thyme. Drying temperature can be affected on the total antioxidant activity because most of antioxidant compounds are phenolic compounds that influence by temperature (Table 2).

Table 2. The effect of drying temperature on antioxidant activity of thyme oil extracts.

Plant extract	IC50 Values ($\mu\text{L}\cdot\text{mL}^{-1}$)*
Fresh thyme	4.9 ± 0.2
Dried thyme in shadow	5.4 ± 0.5

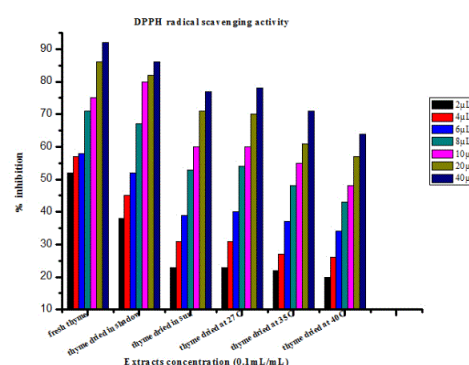


Figure 2. Effect of drying temperature on thyme oil extracts to the% inhibition of DPPH radical scavenging activity.

FRAP method

The total antioxidant potential of sample was determined using a ferric reducing ability of plasma FRAP assay of Benzie and Strain [25]. The FRAP method was employed to estimate the antioxidant capacity of the samples in vitro. All the extracts showed different range of extent of antioxidant activity and this can be related to the high amounts of flavonoids and phenolic compounds in extracts [26].

Dried thyme under sun light	6.9 ± 0.6
Dried thyme at 27°C	7.7 ± 0.4
Dried thyme at 35°C	8.9 ± 0.6
Dried thyme at 40°C	13 ± 0.8
*Means of five determinations ± standard deviation	

The results are calculated reference to ascorbic, gallic and BHT standard curves (Figure 3). Figure 4 shows that the total antioxidant activity of plant extract using values calculated via FRAP method. Effect of drying temperature of thyme oil was noticed in variation of antioxidant activity. The antioxidant activity decrease with increasing the temperature. Fresh thyme shows the highest values of antioxidant activity (0.014

BHTE.mL⁻¹, 0.0064 AAE.mL and 0.0062 GAE.mL⁻¹) of plant extract. Dried thyme at 27°C (0.0091 BHTE.mL⁻¹, 0.0043 AAE.mL⁻¹ and 0.0042 GAE.mL⁻¹ of plant extract) whereas dried thyme at 40°C shows the lowest value of antioxidant activity (0.0042 BHTE.mL⁻¹, 0.0019 AAE.mL⁻¹ and 0.0018 GAE.mL⁻¹) of plant extract [27].

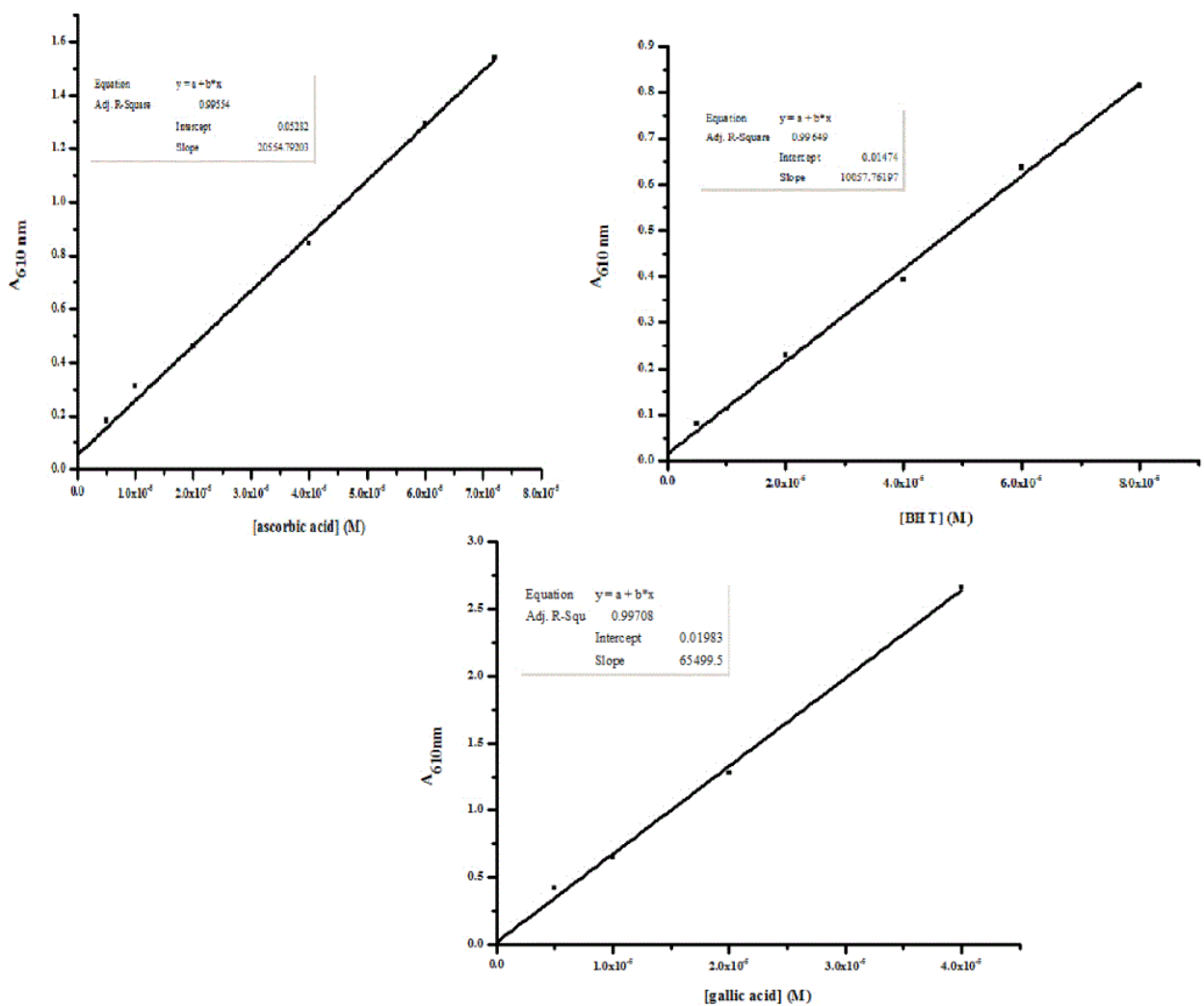


Figure 3. Calibration curve of a: ascorbic acid, b: gallic acid and c: BHT. In acetate buffer pH=3.6, after 30 min at 37°C with max=593 nm.

Conclusions

The results of the present study are clarified that drying temperature breakdown the antioxidant activity of thyme plant. The optimum drying condition was in shadow better than in

sun or drying in oven. The drying temperature more than 35°C led to lower antioxidant activity for thyme plant.

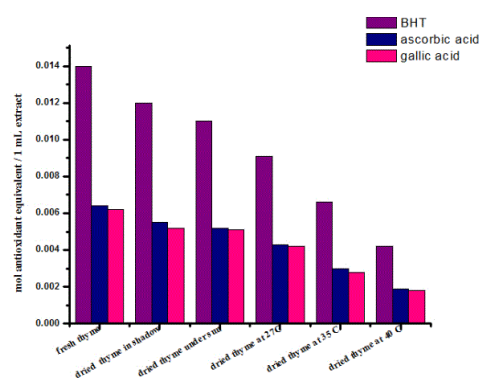


Figure 4. Effect of drying temperature on thyme oil extracts using FRAP calculated values using standard antioxidants (BHT, ascorbic acid and gallic acid).

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