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# Phenolic content and antioxidant properties of selected medicinal and culinary herbs under different temperatures

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ARTICLE INFORMATION	Abstract	
Article History Submitted: 22 Mar 2020 Accepted: 16 Apr 2020	Medicinal and culinary herbs have been used since a long time ago in foo to enhance flavor, color, aroma as well as to increase self-life. Herbs ar rich in numerous bioactive compounds and possess a wide range of ther	
First online: 09 May 2020 Academic Editor Md Abdul Hannan hannanbmb@bau edu bd	peutic properties. This study was conducted to analyze phenolic contents (total polyphenols, phenolic acids and flavonoids contents) and antioxidant activity (DPPH free radical scavenging activity) of selected medicinal and culinary herbs (oregano, basil, coriander, rosemary and thyme) as exposed to different water temperature (27 °C and 100 °C). From this study, the highest total polymbox of a phenolic acida contents ware recorded from 100 °C.	
*Corresponding Author Mansor Hakiman mhakiman@upm.edu.my	<ul> <li>oregano and thyme extracts with 3.79 and 7.06 mg GAE gDW<sup>-1</sup>, respectively. Meanwhile, the highest total flavonoids content was recorded from the 100 °C oregano extract (3.12 mg QE gDW<sup>-1</sup>) and the highest DPPH free radical scavenging activity was exhibited from 100 °C rosemary extract (3.72 mg TE gDW<sup>-1</sup>). Hence, it is suggesting that herbs used in this study were able to withstand high temperature and suitable to use for cooking.</li> <li>Keywords: Culinary herbs, polyphenol, phenolic acid, flavonoid, DPPH</li> </ul>	

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# 1 Introduction

Plants are the major source of bioactive compounds and natural antioxidants. Among all the type of plants, herbs are one of the primary producers of a wide range of bioactive compounds with numerous therapeutic potentials (Yashin et al., 2017). Herbs also have been used extensively in culinary to enhance flavor, aroma, color and improve properties of organoleptic. Besides that, herbs also are widely used in food preservation and production of medicine. As part of human diet, herbs are consumed to provide antioxidants (Embuscado, 2019). Antioxidant is a compound that delays or inhibits the formation of free radicals which causes initiation or propagation of oxidative chain reactions in human body. The high concentration of free radicals in human body causes significant damage to human cells and tissues. As a result, chronic diseases such as cardiovascular disease and cancer can occur. To inhibit the free radicals, antioxidants will neutralize the free radicals by donating their electron (Bratovcic, 2020). Nowadays, synthetic

antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in food as an additive. However, the safety and side effects of these synthetic antioxidants are still not clear (Mohajer et al., 2019). Hence, the uses of natural antioxidants from plant are preferable due to scientifically prove with very low or no side effects to human (Xu et al., 2017). Different species of herbs will exhibit different amount of phenolics content and antioxidant activity. This present study is aimed to analyze the phenolics content and antioxidant activity of selected medicinal and culinary herbs after exposing to different water temperature.

# 2 Materials and Methods

# 2.1 Processing of plant materials

The dried leaves of oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), coriander (*Coriandrum sativum*), rosemary (*Salvia rosmarinus*) and thyme (*Thymus vul-*

garis) were bought from local grocery store. The extraction procedure of dried leaves was conducted following the method employed by Haida et al. (2019) with minor modification. Briefly, the dried leaves were blended using a kitchen blender. In the 50 mL vial that was covered with an aluminium foil, 0.25 g of powdered sample and 12.5 mL of ambient water (27 °C) were added into the vial. The vial was placed on an orbital shaker for an hour in the dark. The sample was filtered using filter paper and the extract was used for further analysis. The same procedure was repeated by replacing ambient water with boiling water.

### 2.2 Quantification of phenolic contents

Total polyphenols content was conducted according to the method employed by Marinova et al. (2005). In the test tubes, a total of 50 µL extracts and ten times diluted Folin-Ciocalteu reagent (1.25 mL) were added and incubated for 5 min. After incubation period, 1.25 mL of 7% sodium carbonate was added and the reaction mixtures were incubated for an hour at room temperature. The absorbance was measured at 725 nm using uv-vis spectrophotometer. A standard curve of absorbance against different concentrations of gallic acid was used to determine the total polyphenols content in the extract and expressed as mg gallic acid equivalents per gram dry weight of sample (mg GAE gDW<sup>-1</sup>).

Quantification of total phenolic acids content was carried out according to the method described by Singleton and Rossi (1965). A total of 0.5 mL of extracts and 4.5 mL of distilled water were added into test tubes. Then, 0.5 mL of Folin-Ciocalteu reagent was added and incubated for 5 minutes. Subsequently, 5 mL of 7% sodium carbonate was added and the final volume was adjusted to 12.5 mL. The reaction mixtures were incubated at room temperature for 90 min and absorbance was measured at 750 nm. A standard curve of absorbance against different concentrations of gallic acid was used to determine the total phenolic acids content in the extract and expressed as mg gallic acid equivalents per gram dry weight of sample (mg GAE gDW<sup>-1</sup>).

For total flavonoids content analysis, aluminium chloride colorimetric method as described by Marinova et al. (2005) was used. A total of 0.5 mL of extracts and 2 mL of distilled water were added into test tubes. Subsequently, 150  $\mu$ L of 5% sodium nitrite was added and the reaction mixtures were incubated for 5 min. After that, 150  $\mu$ L of 10% aluminium chloride was added. At the sixth minute, 1 mL of 1 M of sodium hydroxide was added. The reaction mixtures were mixed thoroughly and absorbance was measured at 510 nm. The total flavonoids content of extract was determined by constructing a standard curve of absorbance against different concentrations

of quercetin. The total flavonoids content of extract was expressed as mg quercetin equivalents per gram dry weight of sample (mg QE gDW<sup>-1</sup>).

#### 2.3 Antioxidant assay

In order to study the antioxidant properties of sample extract, DPPH free radical scavenging activity was conducted according to the method explained by Wong et al. (2006). The 0.1 mM of DPPH was diluted in methanol and initial absorbance was measured immediately at absorbance 515 nm. For sample extracts, a total of 20 µL of each extract was added into 1.5 mL of 0.1 mM of methanolic DPPH solution. The reaction mixtures were incubated for 30 min at room temperature in the dark and the absorbance was measured. A standard curve of absorbance against different concentrations of trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was constructed and DPPH value was expressed as mg trolox equivalent per gram dry weight of sample (mg TE gD $W^{-1}$ ).

#### 2.4 Statistical analysis

All the experiments were carried out in a Completely Randomized Design (CRD) with three replications for each treatment. All data were analyzed using Analysis of Variance (ANOVA) to compare the significant difference between treatments using Statistical Analysis Software version 9.4 (SAS). The comparison of means was conducted using Duncan's Multiple Range Test (DMRT) at p<0.05.

#### 3 Results and Discussion

The study on total polyphenols content of oregano, basil, coriander, rosemary and thyme are tabulated in Table 1. The total polyphenols content recorded was ranged between 0.65 to 3.79 mg GAE gDW<sup>-1</sup>. The highest total polyphenols content was exhibited from 100 °C of oregano extract with 3.79 mg GAE gDW<sup>-1</sup>. Meanwhile, the lowest total polyphenols content was exhibited from 27 °C of coriander extract with 0.65 mg GAE gDW<sup>-1</sup>. From the results in Table 1, all hot water herbs extract except basil extract were produced higher total polyphenols content compared to ambient water extracts. The polyphenol extracted from plant possess numerous therapeutic properties such as anti-cancer, anti-hypertensive, anti-inflammatory and anti-diabetic (Nile et al., 2017).

Thyme and basil extracts significantly produced the highest total phenolic acid content with 7.06 and 7.03 mg GAE gDW<sup>-1</sup> for the hot water treatment, followed by 27 °C of oregano extract with 6.83 mg GAE gDW<sup>-1</sup>, respectively. Among all the herbs extract, hot and ambient water extracts of rosemary were recorded the lowest total phenolic acid content with

Horb	Antioxidant properties <sup>†</sup>		
	Ambient water (27 °C)	Hot water (100 °C)	
	<i>Total polyphenols content (mg GAE gDW</i> <sup><math>-1</math>)</sup>		
Oregano (Origanum vulgare)	$3.71\pm0.020\mathrm{b}$	$3.79\pm0.035~\mathrm{a}$	
Basil (Ocimum basilicum)	$2.18\pm0.017~\mathrm{f}$	$1.13\pm0.008~\mathrm{h}$	
Coriander (Coriandrum sativum)	$0.65\pm0.013$ j	$0.79\pm0.011~{ m i}$	
Rosemary (Salvia rosmarinus)	$2.00\pm0.014~ m g$	$3.34 \pm 0.011 \text{ d}$	
Thyme ( <i>Thymus vulgaris</i> )	$2.81\pm0.032~{\rm e}$	$3.51\pm0.087~{\rm c}$	
	<i>Total phenolic acids content (mg GAE gDW</i> <sup><math>-1</math></sup> )		
Oregano (Origanum vulgare)	$6.83\pm0.048~\mathrm{b}$	$6.36\pm0.022~\mathrm{c}$	
Basil (Ocimum basilicum)	$4.91\pm0.012~\mathrm{e}$	$7.03\pm0.101a$	
Coriander (Coriandrum sativum)	$4.34\pm0.023~\mathrm{f}$	$4.92\pm0.026~\mathrm{e}$	
Rosemary (Salvia rosmarinus)	$3.61\pm0.063$ h	$3.89 \pm 0.029  ext{ g}$	
Thyme ( <i>Thymus vulgaris</i> )	$5.79 \pm 0.028 \text{ d}$	$7.06\pm0.022~\mathrm{a}$	
	Total flavonoids content (mg QE $gDW^{-1}$ )		
Oregano (Origanum vulgare)	$2.65\pm0.020~\mathrm{b}$	$3.12\pm0.017~\mathrm{a}$	
Basil (Ocimum basilicum)	$1.59\pm0.013~\mathrm{d}$	$2.44\pm0.012~\mathrm{c}$	
Coriander ( <i>Coriandrum sativum</i> )	$0.43\pm0.005$ h	$0.45\pm0.001~\mathrm{h}$	
Rosemary (Salvia rosmarinus)	$0.36\pm0.003~{ m i}$	$0.64\pm0.008~{ m g}$	
Thyme ( <i>Thymus vulgaris</i> )	$0.79\pm0.008~{\rm f}$	$0.85\pm0.003~\mathrm{e}$	
	DPPH activity (mg TE gDW $^{-1}$ )		
Oregano (Origanum vulgare)	$2.97 \pm 0.096 \text{ d}$	$2.63\pm0.023~\mathrm{e}$	
Basil (Ocimum basilicum)	$2.95 \pm 0.013 \ d$	$2.45\pm0.049~\mathrm{f}$	
Coriander (Coriandrum sativum)	$3.33\pm0.014~ m c$	$3.58\pm0.009\mathrm{b}$	
Rosemary (Salvia rosmarinus)	$3.66 \pm 0.009$ a	$3.72 \pm 0.021$ a	
Thyme ( <i>Thymus vulgaris</i> )	$3.68\pm0.007$ a	$3.70\pm0.038~\mathrm{a}$	

Table 1. Total polyphenols, phenolic acids and flavonoids contents, and DPPH activity culinary herbs as exposed to different water temperature

<sup>+</sup> Means followed by the same letter are not significantly different at p<0.05

3.89 and 3.61 mg GAE gDW<sup>-1</sup>. In the previous study by Roby et al. (2013), the methanol extract of thyme produced 8.10 mg GAE gDW<sup>-1</sup> of total phenolics content compare to diethyl ether and hexane extracts. The amount of total phenolics content was slightly higher than this present study. However, it was in agreement with this present study that polar solvent able to extract more phenolics content (Table 1).

Oregano extracts exhibited the highest total flavonoids content with 3.12 and 2.65 mg QE gDW<sup>-1</sup>, followed basil extracts with 2.44 and 1.59 mg QE gDW<sup>-1</sup> at 100 °C and 27 °C, respectively (Table 1). The coriander extract was recorded the lowest total flavonoids content in both 100 °C and 27 °C treatments with 0.45 and 0.43 mg QE gDW<sup>-1</sup>. From the results obtained, coriander, thyme and rosemary were recorded two to three folds lower total flavonoids content compared to oregano and basil extracts. In the previous study by Siregar et al. (2017) on coriander leaves extract, the total flavonoids content recorded was 1.37 mg QE gDW<sup>-1</sup>. The total flavonoids content recorded was three folds higher than this present

study might be due to different extraction protocol conducted. Besides that, the age of plant, climate and pre- and post harvest methods conducted also will largely affect the total flavonoids content (Mele et al., 2018).

The DPPH free radical scavenging activity is one of the most frequently used methods in antioxidant activity analysis. As compared to other antioxidant activity methods, DPPH method has simple procedures and consumes low time and chemicals (Zou et al., 2016). Based on the result in Table 1, the DPPH activity of the herbs extract were ranged between 2.45 to  $3.72 \text{ mg TE gDW}^{-1}$ . From the result, there was no significant difference between the rosemary and thyme extracts on DPPH activity. The rosemary and thyme extracted with 100 °C and 27 °C were significantly exhibited the highest DPPH activity. Meanwhile, the lowest DPPH activity was exhibited from 100 °C basil extract with 2.45 mg TE gDW<sup>-1</sup>. The potential of antioxidant recorded from plant samples depends on the amounts of secondary metabolites present in the plant such as polyphenols, flavonoids, tannins and alkaloids. As the secondary metabolites present are high, the redox properties and ability of the plant sample to scavenge free radicals also will be increased (Kasote et al., 2015).

In the present study, the hot water treatment is more prominent in extracting polyphenols, phenolic acids, flavonoids and also produce higher DPPH activity from herbs sample. According to Al-Farsi and Lee (2008), by increasing the aqueous temperature, it will enhance coefficient of diffusion and phenolics content solubility. By increasing phenolics solubility, it will release bound phenolics in the samples. In addition, increased extraction temperature also will caused rate of decomposition of less soluble antioxidants lower than rate of extraction of thermally stable antioxidants (Liyanapthirana and Shahidi, 2005). Hence, more antioxidants can be extracted from the sample.

### 4 Conclusion

In conclusion, oregano extract was found to possess higher phenolic content and exhibit higher antioxidant activity compare to other herbs in this study. Nowadays, the study on secondary metabolites in plant has gain attention around the world. Plant secondary metabolites from natural resources have high market demands due to awareness to consume healthy food or supplement from plant-based products. In the pharmaceutical industry, secondary metabolites extracted from natural resources are important for medicine production. The findings from this study will be beneficial to pharmaceutical and nutraceutical industries. However, extensive research is needed to maximize the amount of secondary metabolites that can be extracted from the plant sample.

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# **Conflict of Interest**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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