QUANTIFICATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID COMPOUNDS IN SWEET BASIL (*Ocimum basilicum* L.) LEAVES, THROUGH THE OPTIMIZATION OF TEMPERATURE AND CONCENTRATION OF ETHANOL

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ABSTRACT

Sweet basil (*Ocimum basilicum* L.) is a valuable pharmaceutical herb that is rich in antioxidants. Despite this, the success in extracting those compounds lies on the technique used during the extraction processes. The current study was aimed to establish a suitable extraction technique through different levels of temperature and concentrations of ethanol. The study was conducted in Post-Harvest Laboratory of Agriculture Faculty in Universiti Putra Malaysia, Malaysia from July 2019 to February 2020. The mature dried leaves of sweet basil were exposed to three different temperatures (40, 60 and 80°C) and combined with three concentrations of aqueous ethanol solutions (60, 75 and 90%, v/v). The experiment was carried out in 3 X 3 factorial complete randomized design with four replications and three samples per replicate. The highest extraction yield (11.56%) was obtained from the combination of 80°C + 90% aqueous ethanol. Meanwhile, the highest total phenolic content (67.02 mg of GAE/g DE), total flavonoid content (44.70 mg QUE/g DE) and antioxidant activity (66.80% in 100 µg/ml) were obtained from the combination of 80°C + 60% aqueous ethanol. Although the highest extraction yield was obtained from 80°C+90%, it is however, this study was focusing on the phenolic and flavonoid compounds. Hence, it is recommended to extract the sweet basil leaves under 80°C with 60% aqueous ethanol.

Key words: antioxidants; extraction; flavonoid; phenolic; sweet basil

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is known as a royal aromatic herb, as it is rich in numerous phytochemicals (Filip 2017). It is considered as a natural edible source of antioxidant compounds (Patriani et al. 2021). There are more than 200 different phytochemicals are reported in sweet basil (Ghasemzadeh et al. 2016). Among all of the phytochemicals, phenolics and flavonoids are major groups of health beneficial antioxidant compounds (Boyer and Liu 2004; Nyamai et al. 2016; King and Young 1999). These groups of compounds are widely used as food ingredients (Tanna et al. 2019).

The extraction of antioxidant compounds from plants involves the withdrawal of unseen compounds from solid particles of plant materials. Establishing methods for extraction of selected antioxidant properties is necessary to ensure high extraction yield. Conventionally, antioxidants are extracted by using methods such as Soxhlet and maceration (Arceusz et al. 2013; Gallo et al. 2020; Turrini et al. 2019). The main drawback of Soxhlet extraction is time consuming and use of large volume of pure solvent, which is not economical (Arceusz et al. 2013), while maceration is time

consuming and has low efficiency (Zhang et al. 2018). Alternatively, solvent extraction method is assumed as of the most economical (in terms of time and efficiency) and easy in terms of operation. Previous researchers claimed that organic solvent extraction was an effective method for extracting phenolic compounds from plant materials, in addition to the instrument (Dorta et al. 2012; Halim 2020; Khoddami et al. 2013).

However, there are some factors involved in influencing the efficacy of extraction process. Among them, the concentration of solvent and level of temperature are the most critical factors that affected the extraction of antioxidant compounds from plant materials (Espada-Bellido et al. 2017; Najafabadi et al. 2020). There were several recommendations on the concentration of solvent to be used which ranges from 60 to 90% (v/v), depending on species (Cacace and Mazza 2003; Elboughdiri 2018; Ko et al. 2018; Toh et al. 2016; Vongsak et al. 2013; Yu et al. 2005). According to Maulana et al. (2019) and Rafińska et al. (2019), the highest amount of phenolic and flavonoid was obtained using aqueous ethanol solution at 90% and 96% in *Toona sinensis* and *Lepidium sativum* plants, respectively. Do et al. (2014) extracted antioxidant compounds from *Limnophila aromatica* with 100% ethanol recorded to receive the optimum amount of total phenolic (40.5 mg of GAE/g of DE), total content of flavonoid (31.11 mg of QUE/g of DE) with highest antioxidant activity (90%). As there is such a wide range of solvent concentrations, it is necessary to test different solvent concentrations on a given species. Similarly, different authors have reported extracting antioxidant compounds at temperature interval of 40 to 80°C (Alberti et al. 2014; Bubalo et al. 2016; Casagrande et al. 2018; Setyaningsih et al. 2019; Sumere et al. 2018; Spigno et al. 2007; Wang et al. 2013). Even for the same plant part (leaf) under same solvent concentration (75% aqueous ethanol solution), the optimum level of temperature for extraction of maximum phenolic compounds from lemon grass (*Cympopogon citratus*) and rosemary (*Rosmarinus officinal*) were 25°C and 75°C, respectively (Juntachote et al. 2006).

Overall, a few publications optimized factors such as temperature and concentration of aqueous ethanol solution for extracting antioxidants from herbal leaves and very few focused on sweet basil. For instance, Teofilović et al. (2017) suggested 96% aqueous ethanol solution and Vidović et al. (2012) established combination of 75.33ºC and 73.66% aqueous ethanol solution; however, the first one did not evaluate temperature and as for the second one, it's difficult for the industry to adjust temperature in decimal points (75.33 ºC). Therefore, this research aimed to determine the suitable temperature and concentration of aqueous ethanol solution to obtain the optimum amount of phenolics and flavonoid compounds from sweet basil leaves.

MATERIAL AND METHODS

Treatments and experimental design. Sweet basil in the form of dry leaf powder were extracted to determine the percentage of extraction yield, total phenolic content, total phenolic yield, total flavonoid content, total flavonoid yield and antioxidant activity by using different temperatures and concentrations of aqueous ethanol solution. The leaves samples were extracted at three different temperatures which were 40, 60 and 80°C and combined with three concentrations of aqueous ethanol solution which were 60, 75 and 90%, v/v. Samples were arranged in three batches and the extractions were performed for three times at 40, 60 and 80°C, respectively. This study was organized in 2-factorial Complete Randomized Design (CRD) by means of four replications and three units per replicate. The study was conducted at Postharvest Laboratory, Crop Science Department, Faculty of Agriculture, Universiti Putra Malaysia**.**

Preparation of plant materials. The leaves of sweet basil were collected from the plants at the age of 60 days after planting. Fresh leaves were separated from stems and washed to remove dust and unwanted substances. The leaves were then placed in an envelope (Manila Envelope, size: 28 cm X 33 cm), and dried using an electronic oven with air renewal circulations (Schutzart DIN 40050 – IP 20,

Memmert, model: UsLM 500) at 50°C (Rocha and Melo 2011) for 7 days. Dried leaf samples were ground in fine particles using electronic grinder (Culatti, Nr. 10318194, Model: MFC) to increase the contact of sample particles with the solvent (Barros et al. 2013). The powdered samples were then placed in small plastic containers and prepared for the extraction.

Extraction. In each observation, 10 g of leaf powder sample was placed into a 250 ml conical flask and aqueous ethanol solution was added at the concentrations of 60, 75 or 90%, v/v at 100 ml for each treatment. After that, the flasks were placed in water-bath (model 760, Schutzart DIN 40050 – IP 20, Memmert, Germany) and subjected to different levels of temperatures (40, 60 and 80°C). Each temperature treatment was set for 90 minutes. The mixture was then filtered using filter pepper (Whatman No. 1, GE Healthcare UK). These procedures were repeated twice in order to make sure that all the targeted compounds were extracted from the leaves. The liquid extract from first and second extractions were combined and proceeded to the next procedure.

The liquid extract was placed in a round flask of 500 ml and placed to an Eyela rotary evaporator (Model: CCA-1111 CE, China) at speed of 45 rpm with water-bath temperature of 40°C and evaporation time of 30±10 minutes to evaporate the solvent. The dry extract was then collected from the flask and weighed using a digital balance (Mettler Toledo, Model: B303-S, Switzerland) and placed into an air tight glass vial before being stored at –20°C for further investigations.

Preparation of chemicals. The analytically graded chemicals and reagents were used in this research. A total of 3.6 ml Folic-Ciocalteu reagent was mixed in 32.4 ml of distilled water to attain 36 ml of 10-fold solution. Sodium carbonate at a concentration of 7.5% was prepared by melting 2.7 g of sodium carbonate (Na2CO3, purity > 99.5%) in 36 ml of distilled water using a magnetic stirrer (FAVORIT, Model: HS0707V2, Serial No: 7306) for 5 minutes. Stock solution of gallic acid was prepared by melting 10 mg gallic acid (C7H6O5.H2O, purity > 99.5%) in 10 ml absolute methanol.

For the analysis of total flavonoid compounds (TFC), 28.8 g of sodium nitrite ($NaNO_2$, purity > 98%) was placed into 144 ml distilled water and melted using magnetic stirrer for 5 minutes. Aluminum chloride at a ratio of 1:10 (w/v) solution was prepared by melting 1.08 g of aluminum chloride ($AlCl_3.6H_2O$, purity > 97%) in 10.8 ml of distilled water using a stirrer for 5 minutes. Also, 72 ml of 1-Mole sodium hydroxide solution was prepared by melting 2.88 g of sodium hydroxide (NaOH, M.W = 40.00 purity > 99%) in 72 ml of distilled water with a magnetic stirrer for 5 minutes. Quercetin stock solution was obtained by melting 5 mg of quercetin (Chem Faces, Cat. No: CFN99272, purity > 98%) in 5 ml of absolute methanol.

In order to evaluate antioxidant activities of the extract solutions, diphenyl-1-picrylhydrayl or DPPH, at 0.1 milli mole in a volume of 36 ml was prepared by melting (1.404 mg DPPH) in 36 ml of absolute methanol using a stirrer for 10 minutes.

Determination of extraction yield. The extraction yield (EY) was measured after the evaporation of solvent from the sample. The dried extract was collected from the rotary flask and weighed using digital balance (Mettler Toledo, Model: B303-S, Switzerland). The same procedures were repeated for all samples. Extraction yield was calculated according to the method developed by Izadiyan and Hemmateenejad (2016) using equation (1), and expressed in percentage.

$$\text{Extraction yield (\%)} = \frac{W_1}{W_2} \times 100 \qquad (1)$$

Where:
W1: is weight of extract after solvent evaporation (g)
W2: is weight of the leaf powder before extraction (g)

Determination of total phenolic content. The determination of total phenolic content (TPC) was carried out according to Ghasemzadeh et al. (2016) with minor modifications. A total of 2 mg dried extract was melted into 1 ml of absolute methanol and mixed well using an orbital shaker (Lab dancer, Model: 3365000, IKA) at a speed of 3,000 rpm for 1 minute. Then, from the solution 200 µL was taken and put in a test tube for analysis. After 1-minute, 1 ml of Folin-Ciocalteu reagent (10 fold) was added in the test tube and mixed well using the orbital shaker for 1-minute followed by the incubation for 10 minutes at total darkness in 25°C. The mixture was then added with 1 ml sodium carbonate (7.5%) and mixed using the orbital shaker at a speed of 3,000 rpm for 1 minute followed by incubation period for 30 minutes at total darkness at a temperature of 25°C. After incubation, the mixture was distributed in micro plate in triplicate and the absorbance was read at 765 nm using a spectrophotometer (Thermo Scientific, Model: 1510, Serial No: 1510-02520C, Fisher Scientific, Malaysia).

Eight different concentrations of gallic acid including 0, 25, 50, 75, 100, 125, 150 and 175 µg/ml were subjected to spectrophotometer at 765 nm for establishing calibration equation. Finally, the content of total phenolic of the sample was calculated according to the method of Genwali et al. (2013), using equation (2) and expressed as milligram of gallic acid equivalent per gram of dry extract (mg of GAE/g of DE).

$$TPC=C*V/M \quad (2)$$

Where:
C: is the concentration of gallic acid from calibration equation (mg/ml)
V: is the volume of tested sample (ml)
M: is the mass of the tested sample (g)

Determination of total phenolic yield. The total phenolic yield (TPY) was calculated to determine the precise amount of total phenolic compounds extracted from the samples. Considering that total phenolic yield could be affected by the extraction yield; TPY was calculated using the equation (3) and stated as TPY in milligram of gallic acid equivalent per 100 grams of dry weight of sweet basil leaves or TPY mg of GAE/100 g of DW.

$$TPY = TPC*EY \quad (3)$$

Where:
TPY: is the total phenolic yield (mg of GAE/100 g of DW)
TPC: is the total phenolic content (mg of GAE/ g of DE)
EY: is the extraction yield (%)

Determination of total flavonoid content. The total amount of flavonoid content (TFC) was determined using the method described by Ghasemzadeh et al. (2016) with minor modification. Dry extract (2 mg) was mixed in 1 ml of absolute methanol and mixed well using the orbital shaker at a speed of 3,000 rpm for 1 minute. The mixture was then poured into a glass test tube and mixed with 4 ml of sodium nitrite solution (1:5, w/v) using the orbital shaker at a speed of 3,000 rpm for 1 minute. The solution was incubated in total darkness at a temperature of 25 °C for 6 minutes. After 6 minutes, a total of 0.3 ml of aluminum chloride solution (1:10, w/v) was added to the solution and mixed well using the orbital shaker at a speed of 3,000 rpm for 1 minute. To complete the reaction, the solution was incubated for another 6 minutes under the same conditions as before. After 6 minutes, 2 ml of 1 mole sodium hydroxide was added following 10 minutes' incubation at 25°C in total darkness. The sample was replicated for three times into a micro plate and placed in the spectrophotometer, where the absorbance was read at 510 nm. For establishing calibration equation, nine different quercetin concentrations including 0, 40, 80, 120, 160, 200, 240, 280 and 320 µg/ml were introduced to the spectrophotometer at 510 nm and the absorbance was recorded. Finally, the total flavonoid content

was calculated by using the equation (4) and expressed as milligram of quercetin equivalent per gram of dry extract (mg of QUE/g of DE).

$$TFC = C*V/M \quad (4)$$

Where:
C: is the concentration of quercetin from calibration equation (mg/ml)
V: is the volume of sample (ml)
M: is the mass of the sample (g)

Determination of total flavonoid yield. To determine the exact amount of total flavonoid compounds obtained from the samples, total flavonoid yield (TFY) was calculated (Hanudin et al. 2012). Taking this into consideration, total flavonoid yield could be affected by the extraction yield. TFY was calculated using the equation (5) and expressed as total flavonoid yield in milligram of quercetin equivalent per 100 grams of dry weight of sweet basil leaves or TFY mg of QUE/100 g of DW.

$$TFY = TFC*EY \quad (5)$$

Where:
TFY: is the total flavonoid yield (mg of QUE/100 g of DW)
TFC: is the total flavonoid content (mg of QUE/ g of DE)
EY: is the extraction yield (%)

Determination of antioxidant activity. The antioxidant potential of leaf extract was performed using method of Aadesariya et al. (2017) with minor modifications. Dry extract (0.1 mg) was melted in 1 ml of absolute methanol and mixed well using the electrical orbital shaker at a speed of 3,000 rpm for 1 minute. The mixture was then placed into a glass test tube. DPPH (0.1 mM, 1 ml) was added and mixed well using the electronic orbital shaker with speed of 3,000 rpm for 1-minute. The mixture was incubated for 30 minutes in darkness at a temperature of 25°C. At the same time, DPPH (0.1 mM, 1 ml) alone was used as a controlling variable. The absorbance of both sample and control was read at 517 nm wavelength using a spectrophotometer. Finally, antioxidant activity of the extract was calculated using the equation (6) and presented as percentage of inhibition.

$$\text{DPPH Inhibition \%} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \quad (6)$$

Where:
A control: absorbance value of control
A test: absorbance value of sample

RESULTS AND DISCUSSION

Extraction yield. The extraction yield was influenced considerably by the interaction of different temperatures and concentrations of aqueous ethanol solution. It was increased significantly, parallel with the increase in temperature and concentration of aqueous ethanol solution (Table 1). The lowest extraction yield was recorded from the combination of 60% aqueous ethanol solution + 40°C which was 5.94% only. Under 60% of aqueous ethanol solution, the extraction yield was increased to 8.08% and 10.34% as the temperature was increased to 60°C and 80°C, respectively. As the concentration of aqueous ethanol solution was increased to 75%, the same pattern was observed as in 60% aqueous ethanol solution, where there was an increased in extraction yield together with the increased of temperature. The extraction yield was increased from 6.41% to 10.39% as the temperature was increased from 40°C to 80°C under 75% aqueous ethanol solution. The maximum extraction yield was obtained from the use of the highest concentration of aqueous ethanol solution (90%) at 80°C.

Table 1. Effect of temperature and concentration of aqueous ethanol solution on extraction yield.

	Extraction yield (%)			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60%**	**75%**	**90%**	**Means**
40	5.94^{g}	6.41^{f}	7.42^{e}	6.59^{C}
60	8.08^{d}	9.03^{c}	9.17^{c}	8.76^{B}
80	10.34^{b}	10.39^{b}	11.56^{a}	10.77^{A}
Means	8.12^{C}	8.61^{B}	9.38^{A}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Based on the results, both factors interacted with each other and positively influenced the extraction yield from dried leaves of sweet basil. Temperature is an important factor in enhancing soluble activities of plant materials during extraction (Efthymiopoulos et al. 2018; Silva et al. 2007; Spigno and De-Faveri 2007) as proven in this study. The mechanism behind stimulation of extraction process using varying temperatures can be attributed to several factors. The adjustment of temperature during the extraction is used to weaken the cell wall, making it easier for the solvent to enter the cell and extract the phytochemical constituents (Esclapez et al. 2011; Kushwaha et al. 2018). Dahmoune et al. (2015) The mechanism in which temperature stimulates the extraction process of phytochemicals is by decreasing the viscosity of solvent molecules (Dahmoune et al. 2015). A previous report revealed that temperature has the potential to destroy cells of plant material by increasing vapour pressure inside the cells, which leads to improved extraction (Zhang et al. (2008). Although high temperature is needed to extract the phytochemicals, it is however dependent on the type and concentration of solvent used. A low amount of extract will be obtained if the concentration of aqueous ethanol used is below 71% at temperature above 74°C in *Ilex kudingcha* (Sun et al. 2011).

Apart from this, aqueous ethanol solution at high concentration could enter the cells and leach the contents. The mechanism behind stimulation of extraction yield by using aqueous ethanol solution could be related to the ability of aqueous ethanol solution in making plant cells larger in order to increase contact surface between solvent and solute sample (Dahmoune et al. 2015). From this present study, the increase in the concentration of aqueous ethanol solution showed positive influence in solubility of phytochemicals in the form of solid particles of sweet basil dry leaves. The result of extraction yield in this study is consistent with an earlier study, where high percent extraction yield was obtained by combining high temperature with high concentration of solvent in Iranian sweet basil accessions (Izadiyan et al. 2016).

Total phenolic content. Data on the effect of different temperatures and concentrations of aqueous ethanol solution on TPC is presented in (Table 2). It was found that the interaction of temperature and aqueous ethanol's concentration had a significant influence on TPC. Extraction done at temperature of 40°C showed the lowest amount of TPC extracted from sweet basil leaves, particularly when extracted with 60% aqueous ethanol solution which was only 28.59 mg of GAE/g of DE. No significant increase was detected when the concentration of aqueous ethanol solution was increased to 75% under the same temperature. By increasing the concentration of aqueous ethanol solution to 90%, the amount of TPC extracted increased sharply at 48.90 mg of GAE/g of DE.

Under 60°C, increasing the aqueous ethanol solution concentration from 60% to 75% and 90%, resulted in a significant increase in TPC from 46.60 mg of GAE/g of DE to 49.52 mg of GAE/g of DE and 51.88 mg of GAE/g of DE, respectively. In contrast, under 80°C, TPC was significantly decreased from 67.02 mg of GAE/g of DE to 58.25 mg of GAE/g of DE and 55.18 mg of GAE/g of

DE by increasing aqueous ethanol solution concentration from 60% to 75% and 90%, respectively. The lowest and highest TPC were obtained through the combination of 40°C + 60% aqueous ethanol solution and 80°C + 60% aqueous ethanol solution which were 28.59 mg of GAE/g of DE and 67.02 mg of GAE/g of DE, respectively.

Table 2. Effect of temperature and concentration of aqueous ethanol solution on total phenolic content.

	Total Phenolic Content (mg of GAE/g of DE)			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60**	**75**	**90**	**Means**
40	28.59^{g}	29.36^{g}	48.90^{e}	35.61^{C}
60	46.60^{f}	49.52^{e}	51.88^{d}	49.33^{B}
80	67.02^{a}	58.25^{b}	55.18^{c}	60.15^{A}
Means	47.40^{B}	45.71^{C}	51.99^{A}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

The results revealed that the maximum amount of phenolic compound was extracted at high temperature with low concentration of aqueous ethanol solution. The efficiency of solvent greatly depends on its polarity, where low concentration of aqueous ethanol solution to water (v/v) results in high polarity. According to Truong (2019), high polar compounds could be easily extracted by the high polar solvents. Since water is highly polar solvent (Zhang et al. 2007), lower concentration of aqueous ethanol solution provides solvent with high polarity potential. Since phenolic compounds are polar, they could be easily recovered by using polar solvent (Zhang et al. 2008). Therefore, it seems that most of the phenolics present in sweet basil leaves were highly polar compounds. This is in agreement with previous researchers who had confirmed the effective recovery of phenolic compounds from plant materials by applying low concentration of aqueous ethanol solution (Kalia et al. 2008; Mith et al. 2016; Ngo et al. 2017).

Total phenolic yield. Total phenolic yield (TPY) was investigated in order to find out which treatment was more sufficient in obtaining larger amounts of phenolic compounds from dry leaves of sweet basil. The results showed significant effect in TPY through the interaction of varying temperatures and aqueous ethanol solution concentrations (Table 3).

Table 3. Effect of temperature and concentration of aqueous ethanol solution on total phenolic yield.

	TPY mg of GAE/100 g of DW			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60%**	**75%**	**90%**	**Means**
40	169.97^{g}	188.40^{g}	362.80^{f}	240.38^{C}
60	376.64^{f}	447.25^{f}	476.20^{d}	433.36^{B}
80	693.50^{a}	605.61^{c}	638.43^{b}	645.84^{A}
Means	413.36^{B}	413.75^{B}	492.47^{A}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Under the temperatures of 40°C and 60°C, increasing the concentration of aqueous ethanol solution from 60% to 75% led to non-significant difference in TPY. By increasing the concentration of aqueous ethanol solution to 90%, under 40°C and 60°C, the extraction of TPY was significantly increased from 188.40 and 447.25 to 362.80 and 476.20 mg of GAE/100 g of DW, respectively. Under 80°C, increasing the aqueous ethanol concentration from 60% to 75% and 90% causes a significant decrease in TPY from 693.50 to 605.61, followed by an increase to 638.43 mg of GAE/100 g of DW, respectively.

In comparison to all temperatures used in this study, it was proven that higher temperature positively influenced the extraction of TPY from sweet basil leaves, whereas under all concentrations of 60, 75 and 90% aqueous ethanol solution, TPY significantly increased (Table 3). Overall, the lowest TPY was obtained from the combination of 40°C + 60% and 40°C + 75% aqueous ethanol solution with values of 169.97 and 188.40 mg of GAE/100 g of DW, respectively. The highest TPY was obtained through the combination of 80°C + 60% aqueous ethanol solution which is 693.50 mg of GAE/100 g of DW.

The higher extraction yield of phenolics at the highest temperature could be related to the solvent diffusive enhancement characteristic of temperature, where high temperature decreases the solvent surface tension, viscosity and stimulates diffusion of the solvent to activate it in order to extract phenolic compounds (Ilaiyaraja et al. 2015; Mohamad et al. 2010; Raj et al. 2020).

In this study, the extraction temperature at 80°C was enough to facilitate the recovery of phenolic compounds (Tables 2 and 3). This was in agreement with the extraction of phenolic constituents from citrus peel (Li et al. 2006).

Total flavonoid content. The extraction of total flavonoid content (TFC) in sweet basil leaves was affected by different temperatures and concentrations of aqueous ethanol solution with significant interaction. At 40°C, data on TFC showed a significant increase from 5.71 to 10.16 and 18.19 mg of QUE/g of DE, respectively, when the concentration of aqueous ethanol solution was gradually raised from 60 to 75, and 90% (Table 4). As the extraction was done at 60°C, the amount of TFC extracted was increased tremendously from 6.22 to 29.68 mg of QUE/g of DE at a concentration of 60 to 75% aqueous ethanol solution, respectively. However, there was no further increase of TFC when the aqueous ethanol concentration was at 90%.

Table 4. Effect of temperature and concentrations of aqueous ethanol solution on total flavonoid content.

	TFC mg of QUE/g of DE			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60%**	**75%**	**90%**	**Means**
40	5.71^{f}	10.16^{e}	18.19^{d}	11.35^{C}
60	6.22^{f}	29.68^{c}	29.97^{c}	21.95^{B}
80	44.70^{a}	32.85^{b}	10.35^{e}	29.30^{A}
Means	18.87^{B}	24.23^{A}	19.50^{B}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Similar to phenolics, a higher recovery of flavonoids was observed at combination of higher temperature with lower solvent concentration. This result agrees with previous findings where a maximum recovery of flavonoid compounds was reported at high extraction temperature (79.07°C and 94.66 °C) in *Euphorbia hirta* L. and *Flos populi* plants, respectively (Abu-Bakar et al. 2020; Sheng et al. 2013). The increase in extraction yield of flavonoid compounds could be attributed to a variety of temperature-related effects. It is well understood that higher temperature increased decomposition of dry leaf sample and facilitated the solubility of flavonoids into solvent (Ghitescu et al. 2015; Liu et al. 2010). On the other hand, lower concentration of ethanol to water (60% v/v) in this study was selected as potential solvent for extracting optimum flavonoids from sweet basil dry leaves. A similar trend was reported earlier in *Crinum asiaticum*, *Passiflora* spp and *Cyclocarya paliurus* (Yu et al. 2019; Gomes et al. 2017; Xie et al. 2015). It was also demonstrated that 80% aqueous ethanol solution blocks solubility of flavonoid compounds from plant cells (Yu et al. 2019). This could be due to the fact that increasing the concentration of ethanol in water would decrease solvent polarity. Therefore, this study suggests that the flavonoid compounds in sweet basil leaves are highly polar compounds such as rutin, kampferol, naringenin and quercetin (El-Nahal and Thabet 2012). The lower concentration of aqueous ethanol solution is therefore more sufficient in extracting these compounds compared to higher aqueous ethanol concentrations.

Total flavonoid yield. Total flavonoid yield (TFY) was calculated in order to differentiate whether the highest amount of total flavonoid is reached from the treatment which resulted in the highest dry extract or highest TFC. TFY increased by increasing the concentration of aqueous ethanol solution at low and moderate temperature while at higher temperature the opposite was observed. At 40°C, increasing the concentration of aqueous ethanol solution from 60 to 75 and 90% increased significantly TFY from 33.99 to 65.19 and 135.01 mg QUE/100 g DW, respectively (Table 5). Meanwhile, extraction done at 60°C showed greater strength to extract TFY where it reached 268.05 mg QUE/100 g DW in 75% of aqueous ethanol solution. Further increase in the aqueous ethanol solution concentration did not show a significant increment of TFY under 60°C. Furthermore, the highest TFY was recorded from 60% aqueous ethanol solution at 80°C which was 462.52 mg QUE/100 g DW.

Table 5. Effect of temperature and concentration of aqueous ethanol solution on total flavonoid yield.

	TFY mg of QUE/100 g of DW			
	Aqueous ethanol (%, v/v)			
Temperature (℃)	**60%**	**75%**	**90%**	**Means**
40	33.99^{h}	65.19^{f}	135.01^{d}	78.07^{C}
60	49.95^{g}	268.05^{c}	275.08^{c}	197.69^{B}
80	462.52^{a}	341.52^{b}	119.79^{e}	307.94^{A}
Means	182.16^{B}	224.92^{A}	176.63^{B}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Antioxidant activity. The interaction between temperature and aqueous ethanol solution concentration had a significant influence in the magnitude of extraction of antioxidants (Table 6). Increasing the concentration of aqueous ethanol solution from 60% to 75%, significantly increased the antioxidant activities from 20.71 to 24.55% at 40°C. However, additional increase in the concentration of aqueous ethanol solution to 90% caused in the opposite.

At 60°C, gradual increment in concentration of aqueous ethanol solution from 60 to 75 and 90% led to a significant increase in antioxidant activities from 21.67 to 30.03 and 33.54%, respectively. Interestingly, at higher temperature of 80°C, increasing the concentration of aqueous ethanol solution from 60 to 75 and 90% significantly decreased the antioxidant activity from 66.80 to 42.19 and 40.84%, respectively. Although it showed a different trend, however, the extraction was recorded to be highest under this temperature.

Table 6. Effect of temperature and concentration of aqueous ethanol solution on antioxidant activity.

	Inhibition% (100 µg/ml)			
	Aqueous ethanol (%, v/v)			
Temperature (°C)	**60%**	**75%**	**90%**	**Means**
40	20.71^{g}	24.55^{f}	25.52^{f}	23.59^{C}
60	21.67^{g}	30.03^{e}	33.54^{d}	28.41^{B}
80	66.80^{a}	42.19^{b}	40.84^{c}	49.94^{A}
Means	36.39^{A}	32.26^{C}	33.30^{B}	

In the same column and row, means showed with the same letters are not significantly different at $p < 0.05$ by Fisher's Protected Least Significant Difference test. The capital letter represents the analysis for two-way ANOVA (3 X 3) and the small letter represents the one-way ANOVA analysis.

Altogether, it can be stated that gradual increase of temperature led to an increase in AA, under low concentration of aqueous ethanol solution. Phenolics and flavonoids are considered as main groups of antioxidant compounds in plants (Aryal et al. 2019; Jung et al. 2011; Scapin et al. 2016). Since these two groups of compounds and AA are higher at the same treatment, it is assumed that they are responsible for antioxidant activity of leaf extracts in sweet basil. This is proved by the results of correlation analysis in this study.

Inside any living cells, Reactive Oxygen Species (ROS) are radical compounds that cause the death, damage and degradation of cells and tissues (Chen 2021). These radicals could be removed by having the antioxidant activity in the tissues or cells (Sharma and Tomar 2021). The antioxidant constituents donate the hydrogen in the electron form and act as the radical intermediates; therefore, they are considered as the main ROS scavengers (Jeong et al., 2004). As a result, including antioxidants in foods and medicines can protect cells from damage and reduce the harmful effects of several diseases. Therefore, the increment in antioxidant compounds and their activities could be a great contribution to the pharmaceutical and nutritive industries.

Pearson correlation analysis. All variables were positively correlated with each other (Table 7). In particular, total phenolic and flavonoid contents were positively correlated with the extraction yield at r=0.98 and r=0.57, respectively. Similarly, total yield of phenolic and flavonoid were correlated positively with total content of phenolic and flavonoid at r=0.85 and r=0.64, respectively. Both groups of phenolic and flavonoid compounds shown positive correlation with antioxidant activity of the extract. This is supported by the other researchers who previously revealed positive correlation between phenolic compounds and antioxidant activity in *Bergenia ciliata* (Genwali et al. 2013),

Lantana camara (Mahdi-Pour et al. 2012) and *Lens culinaris* Medik (Giannakoula et al. 2012). Interestingly, the correlation between total flavonoid content and antioxidant activity was stronger (r = 0.96) when compared with correlation between total phenolic content and antioxidant activity (r = 0.78). This suggests that flavonoids present in sweet basil leaves are highly correlated to the expression of antioxidant activity compared to phenolic compounds**.**

Table 7. Pearson correlation of extraction yield, antioxidant compounds and antioxidant activity in response to different temperature and concentrations of aqueous ethanol solution on sweet basil leaf extraction.

Variables	EY	TPC	TPY	TFC	TFY	AA
EY	1					
TPC	0.98**	1				
TPY	0.78**	0.85**	1			
TFC	0.56*	0.65*	0.74*	1		
TFY	0.98**	0.99**	0.84**	0.64*	1	
AA	0.70*	0.78**	0.86**	0.96**	0.78**	1

Significantly and highly significant expressed by * and **, respectively.
EY: extraction yield, TPC: total phenolic content, TPY: total phenolic yield, TFC: total flavonoid content, TFY: total flavonoid yield and AA: antioxidant activity.

CONCLUSION

The combination of optimal temperature and aqueous ethanol solution are important in extracting the total phenolic and flavonoid contents from sweet basil leaves. Both factors must complement each other in order to obtain high extraction efficiency. In particular, the optimal extraction yield for dry extract was obtained by using 90% aqueous ethanol solution at 80°C for 90 minutes. In addition to this, all the tested variables in this study positively correlated with each other. It is recommended to use 60% aqueous ethanol solution at 80°C for 90 minutes to obtain optimum total phenolic and flavonoid contents with optimum antioxidant activities. Future research is needed to identify other phytochemicals in sweet basil leaves under extraction conditions of 80°C using 90% aqueous ethanol solution.

CONFLICT OF INTEREST

We declare that there is no conflict of interest existing related to this work.

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