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Effects of extraction conditions on total phenolic content and antioxidant capacity of the extract from Thai basil (*Ocimum basilicum* var. *thyrsiflora*) leaves

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Abstract
Thai basil (*Ocimum basilicum* var. *thyrsiflora*) is a common herb in many regions with health benefits. In this study, the effects of different extraction conditions (including water/methanol ratio as solvent, temperature, shaking time and sample/solvent ratio) on total phenolic content of the crude extracts of Thai basil leaves were investigated. The results showed that the extraction condition significantly influenced the total phenolic content of the extract. The highest phenolic content was obtained using 75% methanol in water as the solvent with the shaking duration of 35 min at the temperature of 55°C and with 1/100 (w/w) sample-solvent-ratio. Also, the total phenolic and the flavonoid contents of the extract using the optimal condition were 43.6 ± 0.03 mg GAE/g dry weight and 10.2 ± 0.05 mg RE/g dry weight, respectively. The antioxidant capacity of the extract measured as 2,2-diphenyl-picrylhydrazyl (DPPH) scavenging capacity was 38.2 ± 1.1%. As the result, the extract of Thai basil leaves could be used as a good source of phenolic compounds for further studies.

Keywords: thai basil, extraction, total phenolic content, methanol

1. Introduction
Thai basil (*Ocimum basilicum* var. *thyrsiflora*), belonging to the family of Lamiaceae, is originated and widely cultivated in the Asia (Dolatabad *et al*., 2014) [4]. It has long been used in traditional medicine to treat fever and protect the liver and also possesses antibacterial, antifungal and antioxidant activities (Vlase *et al*., 2014) [17]. Its flowering tops and aromatic leaves are also widely used in food industry as flavoring, and perfumery in hair dressing, perfumes, soaps and mouth washes (Maggini *et al*., 2012) [11]. Phenolic compounds are a main class of basil secondary metabolites that are contributed to antioxidant and anti-inflammatory activity of its extracts (Kwee & Niemeyer., 2011) [9].
Among bioactive compounds in Thai basil are phenolics, which are also found in most plants and their products. There are 3 main groups of phenolic compounds, where phenolic acids, flavonoids and tannins are considered as main dietary phenolic compounds. These compounds express antioxidant activity which is due to their ability to donate hydrogen or electron and delocalize the unpaired electron within the aromatic structure. In order to employ these phenolic compounds in practical applications such as food or pharmaceutical applications, it is important to study their extraction conditions to obtain their high amount and antioxidant activity. In fact, the flowers, seeds or stems contain various phenolic compounds, which require different solvents and conditions for their extraction. Besides water, aqueous methanol and ethanol were commonly used in many researches for extraction of bioactive compounds in plant materials. Extraction of antioxidants from plant materials was commonly conducted by using the Soxhlet method, where heated reflux extraction and maceration methods that are conventional procedures (Arceusz *et al*., 2013). Methanol and ethanol have different polarities, which enable to extract different compounds due to their chemical characteristics in various materials (Jayasinghe *et al*., 2003) [8]. Previous studies concluded that methanol was the best solvent to extract phenolic compounds in Akron wheat (Nguyen and Dang, 2017) [13] and basil (Hossain *et al*., 2010; Parisa *et al*., 2016) [5]. Therefore, the objective of this study was to find out an appropriate condition for the extraction of total phenolic content from Thai basil leaves using a mixture of methanol and water as the solvent. Moreover, the antioxidant capacity and flavonoid content of the extract were also investigated.

2. Materials and Methods
2.1 Sample preparation
The chemicals used for the analyses of total phenolic content and antioxidant capacity include methanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, rutin were purchased from Merk or Sigma-Aldrich.
Thai basil was bought from a local market in Ho Chi Minh City, Vietnam. Blanching pre-treatment was employed with the duration of 10 s. After that, the leaves were separated from stems, arranged on trays and put in a cabinet dryer. Before drying, the initial moisture content of Thai basil leaves was determined according to Association of Official Analytical Chemists (AOAC) method (AOAC 2000).

2.2 Extraction of phenolic compounds
The dried leaves were grinded by an IKA A11 grinder and stored in clean bags in a desiccator until analyses. The extraction of basil leaves followed the method described by Pham & Morita (2008) [14]. In general, the phenolic compounds were extracted with different methanol contents, sample/solvent ratios, incubation temperatures and shaking durations. The powder (1 g) was weighed and put into a 250 mL Erlenmeyer flask. Then, 20 mL of solvent with different methanol contents (0, 25, 50, 75 and 100%) in water was

added and mixed in a shaking incubator under room temperature (30ºC) and stirring (at 200 rpm). After that, the mixture was then centrifuged at 5000 rpm for 5 min to collect the supernatant. The residues were re-extracted for two more times to completely extract the phenolic compounds. All supernatants were then combined and adjusted to 60 mL and stored at 4ºC in the fridge until analyses. The next experiments were carried out using the same procedure with the methanol content that gave the highest total phenolic content and respective changes in incubation temperatures (35ºC, 45ºC, 55ºC and 65ºC), shaking duration (10 min, 35 min, 60 min and 75 min) and ratios of sample to solvent (1:20, 1:50, 1:100, 1:150, 1:200 g/mL) for each experiment. Triplicate was applied in each condition.

2.3 Determination of total phenolic content (TPC)

Total phenolic contents in the extracts were determined using the Folin-Ciocalteu reagent (Vuong *et al*., 2014). 300 µL diluted extract was mixed with 300 µL Folin-Ciocalteu reagent. After 2 min, 2.4 mL of 5% sodium carbonate solution was added. The sample was subsequently incubated at 25ºC for 1 h in the dark. The absorbance was measured at 760 nm. Water was used as blank and gallic acid was used as the standard.

2.4 Determination of total flavonoid content (TFC)

The total flavonoid content in the sample extraction was determined using the aluminum chloride colorimetric method of Chang *et al*. (2002) [3] with some minor modifications. 0.5 mL of the extract was mixed with 1.5 mL 95% ethanol, 0.1 mL of aluminum chloride 10%, 0.1 mL of potassium acetate and 2.8 mL of distilled water. The mixture was then incubated at ambient temperature for 30 min. Eventually, the absorbance of the reaction mixture was measured by a spectrophotometer at 415 nm. The standard calibration was made by using rutin solution with the concentrations of 20, 40, 60, 80 and 100 µg/mL.

2.5 DPPH radical scavenging assay

The antioxidant activity was determined using DPPH radical scavenging assay which was described by Huang *et al* (2005) [6]. Firstly, 0.1 mL of the extracts was mixed 3.9 mL of DPPH. Then, the mixture was kept in dark at ambient temperature. After 30 minutes, the absorbance was measured by a spectrophotometer at 515 nm. Blank was made by the mixture of 3.9 mL of DPPH and 0.1 mL of methanol. After all, the scavenging of DPPH was calculated according to the following equation of Pathirana & Shahidi (2007) [10].

$$\%\ DPPH\ scavenging = \left(1 - \frac{A_{sample}}{A_{control}}\right) x\ 100$$

2.6 Statistical analysis

All data were the means of triplicate measurements. The statistical analyses were carried out using the SPSS software (Statistical Package for Social Sciences) version 22.0 with a level of confidence of 95%.

3. Results and discussion

3.1 Effect of different methanol contents on total phenolic content

While fixing the three conditions, namely, solvent volume of 20 mL, incubation at room temperature and shaking time of 20 min, the effect of methanol content in the solvent on total phenolic content (TPC) of the extracts from Thai basil leaves is shown in Fig. 1. The study showed that the TPC extracted from different methanol contents (0, 25, 50 and 75, 100%) were significantly different ($p < 0.05$). There were no significant differences in TPC of the extracts with the methanol contents of 0, 25 and 50%. As a result, the solvent of 75% methanol was considered as the best solvent to extract the maximum TPC (22.3 mg GAE/ g dry weight). In previous research, the solvent of 80% methanol in water was also considered as the best solvent to extract TPC from different basil leaves such as Greek, blue spice and spice (Kwee *et al*., 2011) [9].

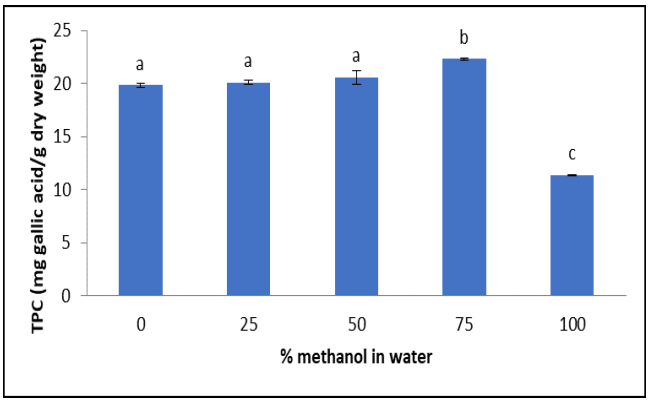

Fig 1: Effect of methanol content in the solvent on total phenolic content of the extract. Different letters (a, b and c) indicate statistically significant differences ($p < 0.05$).

3.2 Effect of incubation temperatures on total phenolic content

Different incubation temperatures significantly affected the total phenolic content of the extracts as shown in Fig. 2. The extraction at 35ºC gave the lower TPC than that at 55ºC (21.88 mg GAE/g dry weight and 24.59 mg GAE/g dry weight, respectively). In general, increasing temperature appropriately can decrease the viscosity and lead to the increase in diffusion coefficients of solutes, including water-soluble phenolic compounds. However, using high temperatures such as 65^{0}C and above might degrade the phenolic compounds in the extracts leading to lower TPC values (Sólym *et al*., 2014). Therefore, the temperature of 55ºC should be used to get the higher TPC of the extractr from thai basil leaves without degradation of the phenolic compounds.

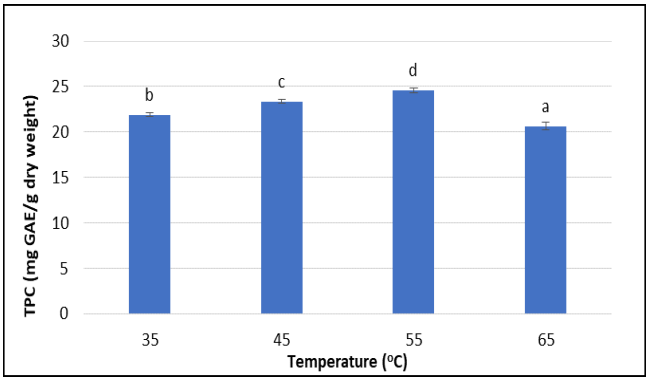

Fig 2: Effect of incubation temperature on total phenolic content of the extract. Different letters (a, b and c) indicate statistically significant differences ($p < 0.05$).

3.3 Effect of shaking durations on total phenolic content

Similarly, by unchanging the two optimal conditions which were methanol content of 75% and the temperature of 55ºC, the effect of shaking time on total phenolic content is shown in Fig. 3. The result shows that the TPCs of the extracts with different shaking durations were significantly different from each other. The highest TPC of the extract was obtained at 35 minutes of shaking (24,6 mg GAE/g DM). At the beginning, the TPC of the extract was lower than those of other samples because the extraction needed more time of shaking in order to obtain more phenolic compounds dissolved in the solvent. However, after the process reached a peak at a specific point of time, the TPC may decrease when the time continued to increase because of antioxidant degradation by several factors such as light, temperature and oxygen. Therefore, the best duration of shaking to get the highest TPC of the extracts was 35 min.

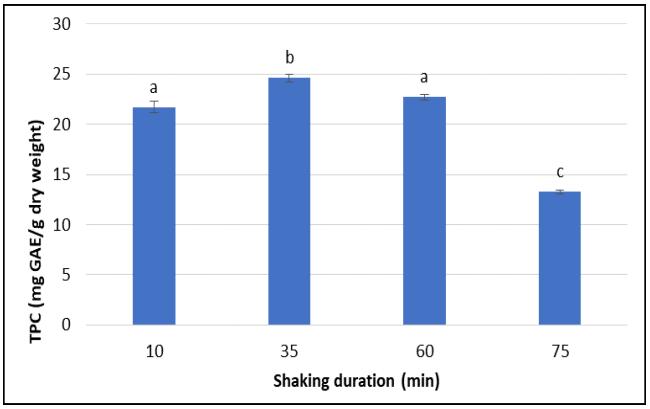

Fig 3: Effect of shaking duration on total phenolic content of the extract. Different letters (a, b and c) indicate statistically significant differences ($p < 0.05$).

3.4 Effect of sample to solvent ratios (g powder/mL) on total phenolic content

The last condition was carried out based on the optimum results of the others. Thus, 75% methanol, 55ºC of temperature and 35 minutes of shaking time were kept constant. The difference in ratio of sample to solvent also affected the total phenolic content of basil leave extracts. Fig. 4 shows that the total phenolic content of sample increased with the ratio of powder/solvent varied from 1/20 to 1/100 (g/mL). This result can be explained by the mass transfer principle. The driving force that drives the mass transfer is the concentration gradient between the solid and solvent explaining the concentration variation between the interior plant cell and the exterior solvent (Nguyen and Dang, 2017) [13]. The previous study also reported that the ratio of 1/100 was optimal for the extraction of total phenolics and flavonoids from basil using an aqueous solution (Parisa *et al*., 2016). In this study, the sample to solvent ratio of 1:100 or 1:150 can be used as the best condition for getting the highest TPC. After all, the appropriate condition to extract the highest TPC from *Ocimum basilicum* var. *thyrsiflora* was 75% methanol in water as solvent, 55ºC as incubation temperature and 35 min as shaking duration and 1:100 as the sample to solvent ratio.

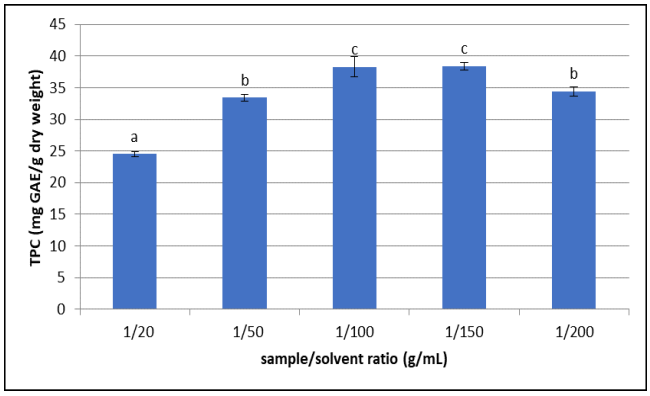

Fig 4: Effect of sample/solvent ratio on total phenolic content of the extract. Different letters (a, b and c) indicate statistically significant differences ($p < 0.05$).

3.5 Bioactive compounds and antioxidant capacity of the Thai basil extract at the optimal extraction condition

The TPC, TFC and DPPH values of the extract from basil leaves using the optimal condition are shown in Table 1. The TPC value was 43.6 ± 0.03 mg GAE/g dry weight, which was higher than the value reported by Hossain *et al*. (2010) [5] which presented the TPCs from 15 different species of basil leaves. This result may be explained by the difference in *Ocimum basilicum* species and the difference in extraction conditions such as solvent, temperature, shaking time and sample to solvent ratio. The results also indicated that the TFC value of the extract was 10.2 ± 0.05 mg RE/g dry weight, which was lower than the values reported by Ghasemzadeh *et al*. (2016) because of different extraction methods. In addition, the DPPH scavenging of the extract was 38.2 ± 1.1 %. One previous study also reported that the DPPH scavenging capacity of *Ocimum basilicum* ranged from 19.23 to 89.46 % (Izadiyan *et al*., 2016) [7].

Table 1: The TPC, TFC and % DPPH values of the extract

Characteristics	Value
TPC	43.6 ± 0.03 mg GAE/g dry weight
TFC	10.2 ± 0,05 mg RE/g dry weight
DPPH	38.2 ± 1.1 %

4. Conclusion

The appropriate extraction condition for the highest total phenolic content of the *Ocimum basilicum* var. *thyrsiflora* extract was determined in this study. The solvent of 75% methanol in water was used effectively to extract bioactive compounds along with other investigated conditions such as the sample-solvent (g/mL) ratio of 1/100, shaking duration

of 35 min and temperature of 55ºC. However, the antioxidant capacity of the extract was relatively low as compared to those reported in a previous study. Therefore, the extract should be purified to obtain higher activity in further investigation.

5. Acknowledgment
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