

Microwave-assisted extraction of total phenolic content (TPC) and antioxidant activities from Yellow bell flowers

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Abstract: -- An efficient microwave –assisted extraction (MAE) technique was developed to extract total phenolics content (TPC) from Yellow bell flowers. The optimal extraction conditions were as follows: methanol concentration 50%v/v; temperature, 60 °C; extraction time, 2 min. At this condition, maximum TPC (0.610 mg gallic acid equivalents/g dry). The condition that maximum total phenolic content was the same as the conditions that maximum antioxidant activity DPPH (0.361 mg TE equivalents/g dry).

Index Terms: - Microwave-assisted extraction, Yellow bells flower, Phenolic content, Antioxidant activity.

I. INTRODUCTION

Tacoma stans (L.) Joss. Ex Knuth or Yellow bell flower, the literature survey reveals that various bioactive compound such as alkaloids, flavonoids, phenols, saponins and quinones. and pharmacological activities such as antioxidant, antimicrobial, antidiabetic, anticancer, antispasmodic, antimicrobial, and antifungal and extensively used in the treatment of diabetes. [1-3] Different techniques have been applied to recover antioxidant compounds from natural sources Different extraction techniques have certain limitations in terms of extraction time, energy, solvent consumption and cost and also influenced by several factors such as the type and concentration of solvent, the solvent/solid ratio, time, temperature etc. The use of microwave-assisted extraction (MAE), compared with the traditional methods, MAE has many advantages, such as shorter time, less solvent, higher extraction rate [4-5] The objective of this research was to screen the independent factors, extraction time, extraction temperature and ethanol concentration on the recovery total phenolic content (TPC) using the MAE from Yellow bell flowers. The antioxidant activity of the obtained extracts was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity.

II. METHOD

A. Material

Yellow bell flowers were collected from Phaisali, Nakhonsawan province of Thailand. The air-dried and

crushed (40 mesh) powder was tightly packed in polyethylene bags and was stored at -4°C for further extraction experiment.

B. Chemical reagents

Sodium carbonate (Na₂CO₃), Folin-Ciocalteu's phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) and Gallic acid was purchased from Sigma-Aldrich Co.

C. Microwave-assisted extraction (MAE)

MAE process was performed using the Microwave Accelerated Reaction System (Model MARS 5®, CEM Matthews, USA) One gram of Yellow bell flower powder was mixed with ethanol ratio 1:10 (w/v). The MAE extraction parameter was ethanol concentration (50-90 %, v/v), extraction time (1-4 min) and temperature (40-70 °C) as follow;

1) The parameter of the effect of extraction temperature are as follows: extraction time 4 min, ethanol concentration 70%. The chosen extraction temperature is 40°C, 60°C and 70°C.

2) The parameter of the effect of ethanol concentration are as follows: extraction time 4 min, extraction temperature, 60°C. The chosen ethanol concentration are 50%, 70% and 90%.

3) The parameter of effect of extraction time are as follows: extraction temperature 60°C, ethanol concentration 50%. The chosen extraction time are 1 min, 2 min and 4 min. After extraction, the mixtures were centrifuged at 3500 rpm for 10 min. The supernatants were collected and stored at -

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20°C until further TPC analysis.

D. Total phenolic content (TPC)

Total phenolics content was quantified using Folin-Ciocalteu reagent using gallic acid as the standard. This method is a modification of method described by Kahkonen et al., 1999 [6] Samples 300 µL were introduced into test tubes; 1.5 mL of Folin-Ciocalteu's reagent and 1.2 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-2300-600, Shimadzu, JP). Standard curves were prepared of gallic acid prepared at concentration of (0.01- 1.0 mg/ml) and the concentration of total phenolics content in flower extract was presented as mg gallic acid equivalent or mg GAE/g dry weight.

E. DPPH radical scavenging assay

The DPPH radical-scavenging assay was used to determine the antioxidant activity of the extract following the method described by Martínez et al., 2012 [7] with some modification. In a test tube containing 300 µL of extract, 4 ml DPPH ethanol solution were added. After 30 min of incubation in the dark at room temperature, the absorbance was measured against a blank (ethanol) at 515 nm was measured (UV-2300-600, Shimadzu, JP). The DPPH capacity was calculated according to the Trolox dissolved in methanol as standard calibration curve and expressed in mg of Trolox equivalents (TE) or mg TE/g dry weight.

III. RESULT

A. Influence of the extraction temperature

Temperature is interrelated with microwave power that controls the quantity of energy converted to heat in the dielectric material. The higher temperature enhances the extraction and reduces the reaction time, but it can also lead to degradation the compound and thus impede the extraction yield. [8] Based on the results presented in Table 1, When temperature increased from 40°C to 60°C amounts of total phenolic content increased from 0.290 to 0.338 mg GAE/g dry at 60 °C and decreased to 0.421 at 70°C . The result indicates that Similarly, Lovic et al.,2017 [9] reported that the highest amounts of TPC were obtained at between 50 and 60 °C from Blackthorn Flowers by MAE. The temperature 60 °C was chosen for the determination of optimal extraction ethanol concentration and extraction time.

Table 1 Total phenolic content and antioxidant capacity of the extracts obtained by microwave-assisted extraction using various at different temperatures.

X ₁	X ₂	X ₃	TPC	DPPH (mg TE/g dry)
70	40	4	0.320	0.290
70	60	4	0.498	0.338
70	70	4	0.421	0.322

			(mg GAE/g dry)	
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70	70	4	0.421	0.322

X1-Ethanol concentration(%v/v), X2- Temperature(°C), X3-Time (min)

B. Influent of the ethanol concentration

Ethanol was selected because of safety for human consumption and polyphenols are highly soluble in polar solvent. It has previously been noted that the efficiency of polyphenol extraction from plant sources is increased in the presence of water in the solvent mixture [10] The result show in Table 2, When ethanol concentration increased from 50% to 70% (v/v), the total phenolic content of the extracts decreased from 0.566 to 0.496 mg GAE/g dry. When ethanol concentration increased to 90% (v/v), the total phenolic content decreased, the total phenolic content was 0.385 mg GAE/g dry. Similarly, Zhange et al.,2008 [11] reported that 50% ethanol extraction solvent gave the best yield of chlorogenic acid. The solvent with 50% ethanol concentration was then chosen for the determination of optimal extraction time.

Table 2 Total phenolic content and antioxidant capacity of the extracts obtained by microwave-assisted extraction using various at different ethanol concentration.

X ₁	X ₂	X ₃	TPC (mg GAE/g dry)	DPPH (mg TE/g dry)
50	60	4	0.566	0.356
70	60	4	0.496	0.340
90	60	4	0.385	0.301

X1-Ethanol concentration(%v/v), X2 -Temperature(°C), X3-Time (min)

C. Influent of the extraction time

The contents of total phenolic from Yellow bell flower at different MAE times are presented in Table 3. The results indicate that the extraction of phenolics was increased with an increase in MAE time the total phenolic content was 0.610 mg GAE/g dry in 2 min. but with the longer exposure of the sample to the microwave irradiation and solvent, the amount of total phenolic content in extracts decreased. The longer exposure of the sample to the solvent and microwave irradiation, similarly, extraction of polyphenols from the waste yarrow dust, and caused the relative decrease of the polyphenols in extracts [12].

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Table 3 Total phenolic content and antioxidant capacity of the extracts obtained by microwave-assisted extraction using various at different time.

X ₁	X ₂	X ₃	TPC (mg GAE/g dry)	DPPH (mg TE/g dry)
50	60	1	0.598	0.357
50	60	2	0.610	0.361
50	60	4	0.558	0.358

X1-Ethanol concentration (%v/v), X2-Temperature(°C), X3-Time (min)

DPPH radical scavenging activity value show similar trend as total phenolic content. (Table 1,2 and 3), the antioxidant activity of the extracts is directly proportional to the total polyphenol content in the extracts. The results, that indicate that conditions that the maximum extraction of total phenolic content are also the same as the conditions that maximum antioxidant activity are in agreement with Milutinovic et al.,2014 [13] reported.

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