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Effect of Extraction factor on Total Phenol content and Antioxidant activity of Coffea

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Abstract: -- **The objective of present work was assessing the optimization of a batch extraction process for phenolic compounds and DPPH from Robusta (Chumphon, Southern of Thailand), Arabica (Doi Chaang northern of Thailand) type of different geographical original and coffee black bean. The effects of extraction solvent concentration and ratio of solvent to solid during extraction process on phenolic compound and antioxidant activity were investigated. The suitable condition for batch extraction were 60% (v/v) by using ethanol as solvent, solvent to solid ratio of 20: 1 (ml/g) during 90 min was the most suitable condition to produce an extract with high content of phenolic compound of green coffee Robusta, Arabica and defect (33.25, 28.12 and 26.17 mg Gallic equivalents/g) and high antioxidant activity of green coffee Robusta, green coffee Arabica and green coffee defect (2-diphenyl-1-picrylhydrazyl (DPPH) of 28.62, 24.67 and 24.82 mg/ml) The extraction results indicated that antioxidant power was highly correlated to the content of phenolic compounds.**

Index Terms: - Coffee Robusta, Coffee Arabica, Coffee defect, antioxidant activity, total phenol, extraction.

I. INTRODUCTION

Coffee is one of the most popular beverages in the world. The two most economically important varieties of coffee plant are the Arabica (60%) and Robusta coffee (40%). That consists of bioactive compound such as 1.2-2.2% caffeine and 6.5-10.0% chlorogenic acid. Species of Coffea or geographical origin influence the content of major compounds in the extract of coffee bean as well as its antioxidant activity.[1] Coffee defect, the beans shriveled, and with the crack too open. Causes include over fermentation, over-ripe cherries, and not enough water during cherry development. Extraction method is the first step in the isolation of phenolic compounds from coffee. Different techniques have been applied to recover antioxidant compound from natural sources including solvent extraction, Ultrasound-assisted extraction, supercritical CO2 extraction [2] and microwave assisted extraction. Among these techniques, solid-liquid extraction is widely employed for antioxidant extraction from natural source. However, the efficiency of the extraction process is affected by several factors such as the type of solvent and the solvent/solid ratio. Thus, it is very important to optimize the extraction conditions in order to maximize the extraction efficiency to each raw material.

Figure 1 Coffee cheery and Green coffee bean

II. MATERIALS AND METHODS

A. Sample materials

Material: Coffee bean (C. Arabica) were collected from (Doi Chaang northern of Thailand) and Coffee bean (C. Robusta) and Coffee Robusta defects were collected from (Chumphon, Southern of Thailand) was dried in an oven at 105 °C for 48 hr. and grinder to green coffee bean powder. Chemical: Gallic acid, ethanol, Folin-Ciocalteu phenol reagent,2,2-diphenyl-1-picrylhydrazyl(DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Sodium Carbonate (Na2CO3) Submit your manuscript electronically for review**.**

Figure 2 A: Green coffee Arabica
B: Green coffee Robusta
C: Green coffee Robusta (Defect)

B. Solvent extraction

The extraction were performed using ethanol concentration (60%) and different condition of solid/solvent ratio (1:10, 1:15, 1:20 and 1:25) and extraction time 90 min with magnetic stirrer. The extract was separated from solid residues by centrifugal (3000 rpm, 5 °C 15 min) and the supernatant was filtered through 180 µm filters. The pooled extracts were distilled on a rotary evaporator at 50 °C under

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reduced pressure (40 millibar) and the product stored at 4 °C.

C. Antioxidant activity

Coffees were extracted with 60% Ethanol ratio (1:10, 1:15, 1:20 and 1:25) on magnetic stirrer for 90 min. The mixture was centrifuged at 3000 rpm, 5 °C 15 min. The extracts were filtered and the supernatant was filtered through 180 µm filters. The pooled extracts were distilled on a rotary evaporator at 50 °C under reduced pressure (40 millibar). The clear extracts were analyzed both for determination of total phenolic and antioxidant activity.

1. Determination of total phenolic content

The total phenolic concentration was measured using the Folin-Ciocalteu method. [3] The extracts (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. Absorbance at 765 nm was measured (UV-1800, Shimadzu, Japan). The results were expressed as mg Gallic acid equivalents (mg GAE/100g dry mass).

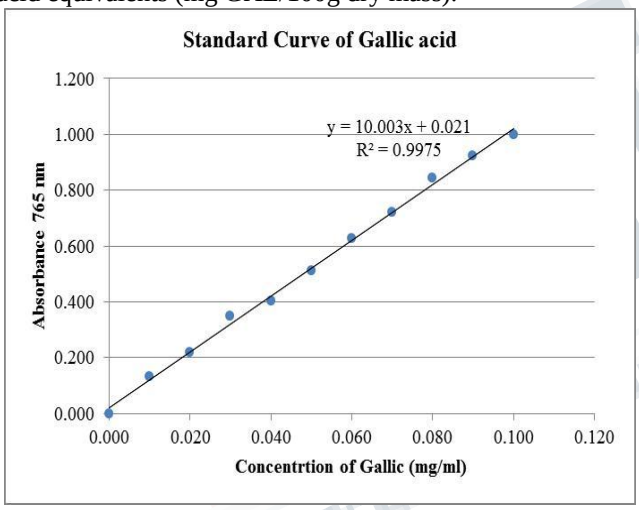

Figure 3 Standard of Gallic acid curve

2 Determination of DPPH radical scavenging activity

The DPPH assay was conducted according to the method of Mahayotpanya C., 2016.[3] A stock solution was prepared by dissolving 0.0240 g DPPH with 100 mL of 99.99% Ethanol. The working solution was obtained by mixing 40 mL stock solution with 240 mL of 99.99% ethanol (ratio of 1:6) to obtain an absorbance of 0.94±0.02 units at 515 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). The extracts (300 µL) were allowed to react with 4 mL of DPPH solution for 30 min in the dark. Then, the absorbance was determined at 515 nm. The results were expressed as mg Trolox equivalents (mgTE/100g dry mass). Percentage inhibition was calculated using equation 1,

Equation 1,

$$\%\,inhibition = \left(\frac{A\text{ of control} - A\text{ of sample}}{A\text{ of control}}\right) x\,100$$

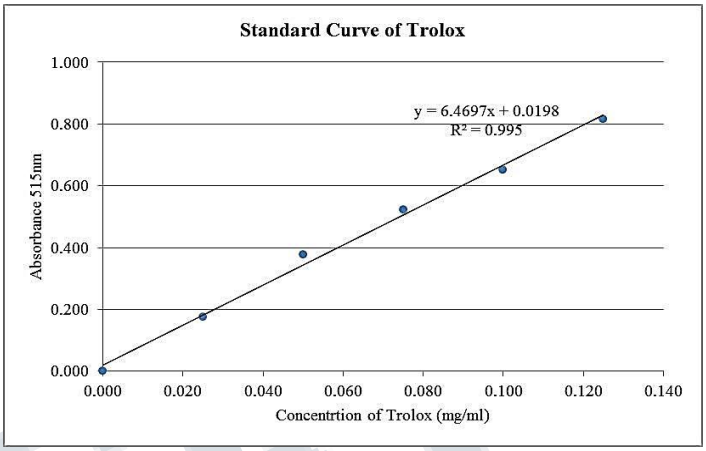

Figure 4 Standard of Trolox curve

III. RESULTS AND DISCUSSION

The type of bean and solid/solvent ratio are key factors in extraction processes, as they affect both the kinetics of phenolic release from the solid matrix and the antioxidant activity of the extract. Therefore, this study consisted in evaluating the effect of these variables, type of bean and solvent/solid ratio on the recovery of antioxidant phenolic compounds from Green coffee. The results obtained in these experiments are shown in Table 1. The extraction results (total phenolic and antioxidant activity), which were improved when the ethanol concentration was decreased, and the solid/solvent ratio were increased. In fact, the best results of total phenolic were achieved in the conditions that used ethanol in concentration of 60% and in a solid/solvent ratio of 1:20 during 90 min (Table 1).

Figure 5 A. Extraction method B. Solvent extraction

Table 1 Experiment condition used to evaluate the effect of process variables (Type of bean and solid/solvent ratio) on the antioxidant activity phenolic compound from coffee

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Arabica, coffee Robusta and coffee defect and antioxidant activity of each solid/solvent ratio

Assay	Process variables		Responses (mg GAE/100 g)	
	Coffee Bean	Solid/solvent ratio (g/ml)	TP	DPPH
1	Arabica	1:10	23.68	14.2
2		1:15	27.69	17.88
3		1:20	28.07	24.66
4		1:25	28.12	24.67
5	Robusta	1:10	31.22	23.87
6		1:15	31.55	27.33
7		1:20	32.96	29.61
8		1:25	33.26	28.62
9	Defect	1:10	22.25	17.41
10		1:15	25.43	21.82
11		1:20	26.18	24.82
12		1:25	24.63	24.83

TP: Total phenol content, DPPH: antioxidant activity

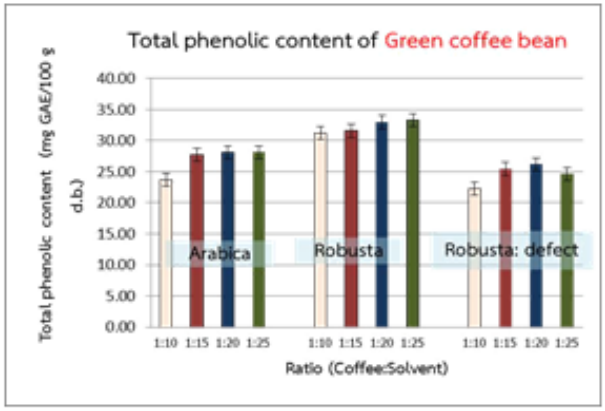

Figure 3Effect of ethanol ratio on phenolic compound

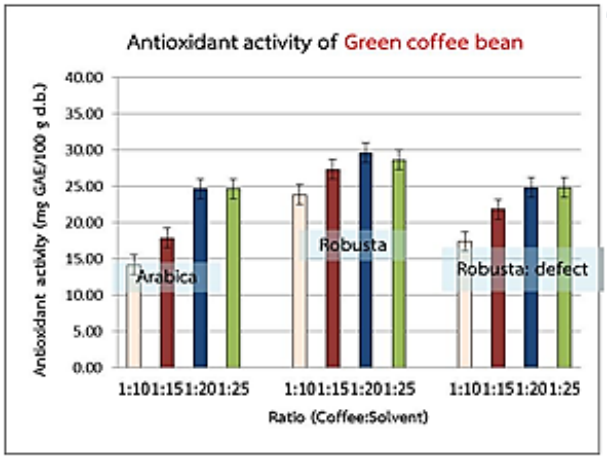

Figure 4 Effect of ethanol ratio on DPPH activity

Green Coffee Robusta provided the highest total phenolic and DPPH antioxidant activity due to high solvent ratio. The suitable condition for batch extraction were 60% (v/v) by using ethanol as solvent, solvent to solid ratio of 20: 1 (ml/g) during 90 min was the most suitable condition to produce an extract with high content of phenolic compound of green coffee Robusta, green coffee Arabica and green coffee defect (33.25, 28.12 and 26.17 mg Gallic equivalents/g) and high antioxidant activity of green coffee Robusta, green coffee Arabica and green coffee defect (DPPH) of coffee bean 28.62, 24.67 and 24.82 mg/ml)

IV. CONCLUSION

The results indicate that the solvent extraction methods are a preliminary technique for phenolic compound and antioxidant extraction from both of Coffee Arabica and Coffee Robusta.The solvent extraction method using the ethanol-water mixture. Furthermore, antioxidant activities of Coffee Robusta extract were higher than Coffee defect and Coffee Arabica extract with the same solvent extraction ratio.

V. ACKNOWLEDGMENT

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