



Optimal condition of cannabis maceration to obtain the high cannabidiol and Δ^9 -tetrahydrocannabinol content

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Abstract: The aim of this work was to optimize a maceration condition of cannabis (*Cannabis sativa* L.). A circumscribed central composite experimental design was applied in this work. Temperature and time were varied from 40-80 °C and 30-90 min, respectively. The three responses (i.e., extraction yield, cannabidiol content, and Δ^9 -tetrahydrocannabinol content) were predicted by computer software. The yield was high when cannabis was macerated using ethanol at high temperature and long duration time. While cannabidiol and Δ^9 -tetrahydrocannabinol content was high when macerating at a low heating temperature and short duration time. The optimal condition provided the simultaneous high of cannabidiol and Δ^9 -tetrahydrocannabinol content was 40 °C for 30 min. The prediction was accurate due to low percent error. This optimal condition could be used as a guide for maceration of cannabis to obtain the extract containing a high content of cannabidiol and Δ^9 -tetrahydrocannabinol.

Key words: *Cannabis sativa*, cannabidiol, Δ^9 -tetrahydrocannabinol, optimization.

INTRODUCTION

Cannabis sativa L. is a plant in Family Cannabaceae. In Thailand, it is categorized as a narcotic drug. Cannabis contains at least 750 identified chemical constituents, among these, 104 compounds are cannabinoids (Radwan et al. 2015). According to the survey from 31 countries in 953 subjects, cannabis is mostly used for the treatment of chronic pain,

anxiety, loss of appetite, depression, and insomnia (Hazeekamp et al. 2013). There are many systematic reviews and meta-analyses reveal that cannabis can be used in several diseases and for symptoms such as cancer-pain (Aviram and Samuelly-Leichtag 2017), chronic non-cancer pain (Aviram and Samuelly-Leichtag 2017, Martin-Sanchez et al. 2009, Stockings et al. 2018a, Whiting et al. 2015), chemotherapy-induced nausea and vomiting (Machado Rocha et al. 2008, Whiting et al. 2015), epilepsy (Pamplona et al. 2018, Stockings et al. 2018b), insomnia (Whiting et al. 2015), neuropathic

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pain (Aviram and Samuelly-Leichtag 2017, Nugent et al. 2017, Ware et al. 2010), spasticity (Whiting et al. 2015), Tourette syndrome (Whiting et al. 2015), and weight loss (Whiting et al. 2015), etc.

Cannabidiol (CBD) and Δ^9 -tetrahydrocannabinol (Δ^9 -THC) are the most well-known cannabis active compounds. Δ^9 -THC is a primary psychoactive compound, while CBD is a non-psychoactive compound. CBD and Δ^9 -THC are derived from their acidic precursors in plants; cannabidiolic acid (CBDA) and Δ^9 -tetrahydrocannabinolic acid (THCA), respectively. The two acidic precursors are both derived from cannabigerolic acid (CBGA), which was converted to CBDA and THCA by CBDA synthase or THCA synthase, respectively. According to CBDA and THCA, they are decarboxylated to CBD and Δ^9 -THC by light exposure, heating, or aging (World Health Organization 2017). There are two major cannabinoid receptors; CB₁ and CB₂ receptors. CB₁ receptors are mostly located in a central nervous system with some peripheral tissues, while CB₂ receptors are mostly located periphery on immune cells and gastrointestinal tract. Δ^9 -THC is a partial agonist to CB₁ and CB₂ receptors, while CBD act as an antagonist of CB₁ and CB₂ receptors agonists with apparently low affinity (Pertwee 2008). The effects of Δ^9 -THC are muscle relaxation, analgesia, and antiemesis. However, it induces psychosis, anxiety, and sedation. CBD is anxiolytic, antipsychotic, and antisedating. CBD can reduce the effect of Δ^9 -THC by inhibition of metabolism of Δ^9 -THC to 11-hydroxytetrahydrocannabinol, a more psychoactive metabolite (Howard et al. 2013). Combination of Δ^9 -THC and CBD can improve the efficacy and tolerability profile of Δ^9 -THC. Nowadays, there are cannabis-based commercial products containing natural cannabinoids; Sativex[®] and Epidiolex[®]. Sativex[®] is an oromucosal spray, each 100 μ L contains 2.7 mg Δ^9 -THC and 2.5 mg CBD. It is used for the treatment of spasticity due to multiple sclerosis (eMC 2018). While Epidiolex[®] is

an oral solution, it contains 100 mg/mL CBD. It is used for treatment of epilepsy associated with Lennox-Gastaut syndrome or Dravet syndrome (FDA 2018).

Extraction is an essential first step in the analysis of chemical compounds in plants. This step is required to extract the desired chemical compounds from plant materials for further use. Proper extraction must be taken to assure the plant chemical compounds are not destroyed during the extraction process (Sasidharan et al. 2010). Furthermore, the proper extraction condition can provide the highest content of active chemical compounds. The aim of this work was to optimize a maceration condition of seized cannabis. A validated high performance liquid chromatography was used for determination of CBD and Δ^9 -THC. A circumscribed central composite experimental design was applied in this work to evaluate the effect of maceration temperature and duration time on extraction yield, CBD content, and Δ^9 -THC content. We expected that the optimal condition obtained from this work could be used as a guide for maceration of cannabis to obtain the extract containing a high content of CBD and Δ^9 -THC.

MATERIALS AND METHODS

MATERIALS

CBD (purity 97.4%, HPLC) and Δ^9 -THC (purity 98.9%, HPLC) isolated from seized cannabis bar were used as a standard marker for analysis of CBD and Δ^9 -THC containing in cannabis extract. They were certified by NMR and GC-MS. Methanol (HPLC grade) was purchased from Honeywell Burdick & Jackson, USA. Absolute ethanol was purchased from QR&C, New Zealand.

CANNABIS SAMPLE AND EXTRACTION PROCEDURE

Seized cannabis bars were obtained from the Narcotics Suppression Bureau with the permission

of Office of the Narcotics Control Board, Thai Food and Drug Administration, Ministry of Public Health. They were deposited at College of Pharmacy, Rangsit University. Seized cannabis bar was cut into small piece, ground, and passed through a 20-mesh sieve.

The 10 g of cannabis powder was added to 250-mL Erlenmeyer flask. Then, absolute ethanol (100 mL) was added. It was macerated under specific temperature and duration time according to the circumscribed central composite experimental design as shown in Table I. The maceration was done using a water bath (WNB 14, Memmert, Germany). After maceration, the filtrate was collected by vacuum filtration. The marc was macerated again using the same condition for an additional two times. The three filtrates were pooled and the solvent was eliminated by a rotary evaporator (Buchi R-100, Büchi Labortechnik AG, Switzerland). Extraction yield data was collected and the extracts were kept in a desiccator until use.

OPTIMIZATION OF MACERATION OF CANNABIS

The circumscribed central composite experimental design was applied in this work. Two factors were varied; temperature (X_1) and duration time (X_2). Temperature and time were varied from 40-80 °C and 30-90 min, respectively. Ten conditions (eight non-center points and two center points) according to the circumscribed central composite experimental design are shown in Table I. Cannabis powders were individually extracted following the ten conditions and the extracts from different conditions were analyzed for CBD and Δ^9 -THC content using high performance liquid chromatography (HPLC). Three responses; extraction yield (Y_1), CBD content in the extract (Y_2), and Δ^9 -THC content in the extract (Y_3) were monitors. The above data was analyzed by Design-Expert version 11.0. Contour plots, response surfaces, and desirability plot were constructed. The equations for prediction of each

TABLE I
Coded and actual value of model conditions of the circumscribed central composite experimental design.

| Condition | Temperature | | Duration time | |
|-----------|-------------|-------------|---------------|--------------|
| | Coded | Actual (°C) | Coded | Actual (min) |
| 1 | -1 | 45.9 | -1 | 38.8 |
| 2 | 1 | 74.1 | -1 | 38.8 |
| 3 | -1 | 45.9 | 1 | 81.2 |
| 4 | 1 | 74.1 | 1 | 81.2 |
| 5 | $-\sqrt{2}$ | 40.0 | 0 | 60.0 |
| 6 | $\sqrt{2}$ | 80.0 | 0 | 60.0 |
| 7 | 0 | 60.0 | $-\sqrt{2}$ | 30.0 |
| 8 | 0 | 60.0 | $\sqrt{2}$ | 90.0 |
| 9 | 0 | 60.0 | 0 | 60.0 |
| 10 | 0 | 60.0 | 0 | 60.0 |

response were reported. In addition, analysis of variance was performed for each response. The correlations between the factors were determined using SPSS version 22. The optimal condition that gave the highest content of CBD and Δ^9 -THC was selected based on desirability value (Bezerra et al. 2008). This optimal condition was used to extract cannabis powder again. The extraction yield, CBD content, and Δ^9 -THC content were reported. The percent error of the prediction was reported for each response. This value was used to confirm the accuracy of the prediction.

QUANTITATIVE ANALYSIS OF CBD AND Δ^9 -THC IN CANNABIS EXTRACT

The analysis of CBD and Δ^9 -THC content was done by HPLC instrument (Agilent 1260 Infinity, Agilent, USA). ACE[®] C18-PFP (250×4.6 mm, i.d., 5 μ m) (Advanced Chromatography Technologies Ltd., UK) connected to guard column (SecurityGuard Cartridge C18, 4.0×3.0 mm, i.d.) (Phenomenex Inc., USA) was used for the isocratic separation. Column temperature was controlled at 25 °C. Mobile phase consisted of water and methanol in a ratio of 10:90 v/v. Mobile phase flow rate was set at 1 mL/min and injection volume was 10 μ L. The

response signal was monitored at 222 nm. The total analysis time for each injection was 25 min.

RESULTS AND DISCUSSION

CBD and Δ^9 -THC in cannabis extract were quantified by reversed-phase HPLC. The HPLC method was validated to ensure the reliability of the analysis. The HPLC chromatograms of CBD, Δ^9 -THC, and cannabis extract are shown in Fig. 1. CBD and Δ^9 -THC were eluted at retention time of 5.1 and 9.8 min, respectively.

Our work found that the extraction yield of the ten conditions was in the range of 18.63-22.39%. Condition 5 and Condition 8 had the lowest and highest extraction yield, respectively. CBD and Δ^9 -THC content in the extract was in the range

of 6.98-8.10% and 25.90-30.67%, respectively. Condition 8 and Condition 5 had the lowest and highest CBD content as well as Δ^9 -THC content, respectively. However, a narrow range of CBD and Δ^9 -THC content in raw material extracted from the ten conditions was observed; 1.49-1.65% and 5.51-6.05%, respectively. Recently, there was a high efficient technique to extract the CBD and Δ^9 -THC such as supercritical fluid extraction (Elkins et al. 2019, Gallo-Molina et al. 2019). However, the extraction of crude extract by maceration was still necessary to concentrate the CBD and Δ^9 -THC yield before supercritical fluid extraction was done.

Contour plots and response surfaces of model conditions of extraction yield, CBD content and Δ^9 -THC content are shown in Fig. 2. They were fitted with linear mathematic models. Increasing

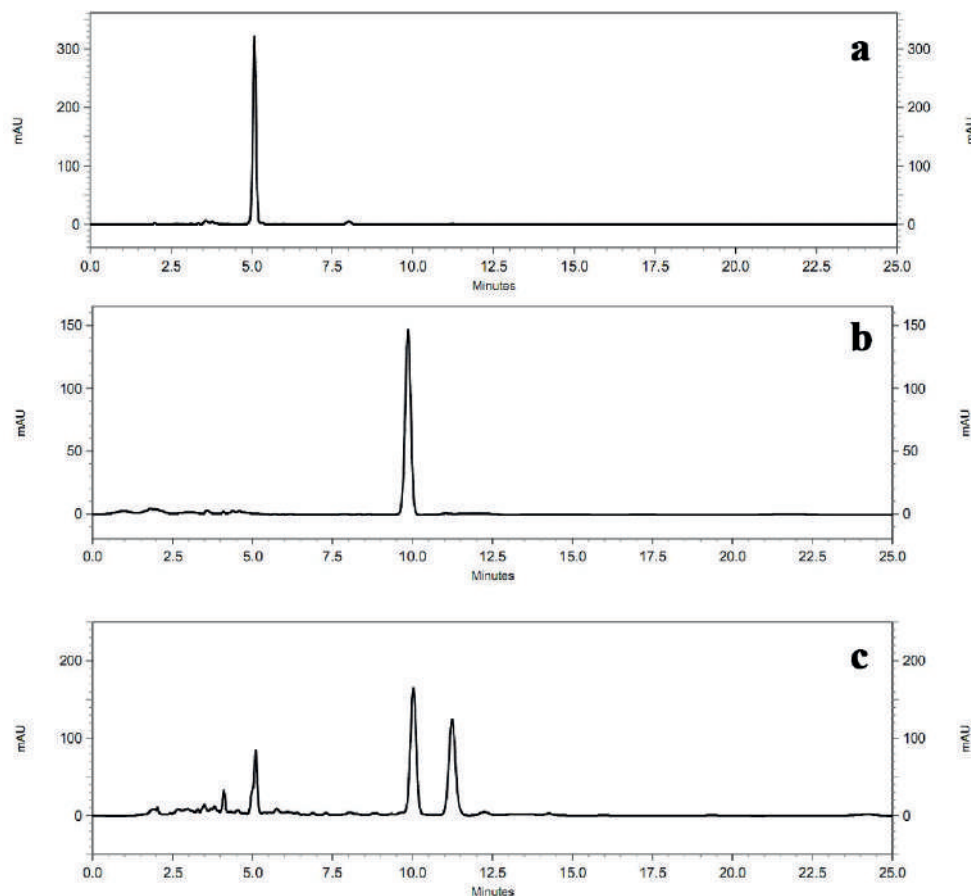


Figure 1 - HPLC chromatograms of (a) CBD (100 $\mu\text{g/mL}$), (b) Δ^9 -THC (100 $\mu\text{g/mL}$), and (c) cannabis extract (500 $\mu\text{g/mL}$).

temperature as well as duration time provided high extraction yield (Fig. 2a). According to CBD and Δ^9 -THC content in the extract, increasing temperature and duration time provided low content of CBD and Δ^9 -THC (Fig. 2b and 2c). The coded and actual equations for prediction of extraction yield, CBD content, and Δ^9 -THC content are shown below. The equations revealed that temperature and time had a positive effect on extraction yield, while they had a negative effect on CBD and Δ^9 -THC content. From the equations, the coefficient of maceration temperature had higher value compared to maceration time, so maceration temperature had a major effect on CBD and Δ^9 -THC content rather than maceration time.

Coded equations

$$Y_1 = 21.00 + 0.76(X_1) + 0.41(X_2) \quad (1)$$

$$Y_2 = 7.52 - 0.30(X_1) - 0.16(X_2) \quad (2)$$

$$Y_3 = 27.84 - 1.15(X_1) - 0.49(X_2) \quad (3)$$

Actual equations

$$\text{Yield} = 16.617 + 0.054(\text{Temp.}) + 0.019(\text{Time}) \quad (4)$$

$$\text{CBD content} = 9.228 - 0.021(\text{Temp.}) - 0.008(\text{Time}) \quad (5)$$

$$\Delta^9\text{-THC content} = 34.122 - 0.082(\text{Temp.}) - 0.023(\text{Time}) \quad (6)$$

Extraction temperature and time were two of several factors that affected the extraction efficiency of active chemical compounds of various plants. Much research has investigated the effect of extraction temperature and extraction time on plant chemical compounds. Yapo et al. (2007) isolated pectin from sugar beet pulp, and found that extraction time had a positive effect on pectin yield compared to extraction temperature. Vergara-Salinas et al. (2012) determined the effect of temperature and time on the polyphenolic content of *Thymus vulgaris*. They reported that the content

of polyphenols was diverse with regard to different extraction temperatures and extraction times. The higher temperature and longer time could decrease the diversity of hydroxycinnamic acids, flavones, flavonols/flavanones, and total polyphenols. The phenolic compounds that mostly extracted at high temperature were 3,4-dihydroxyphenyllactic acid. In case of nonphenolic antioxidant, it was highly extracted at the high temperature and long extraction time. Dent et al. (2013) showed that extraction temperature had a positive effect on mass fractions of total polyphenols, rosmarinic acid, and luteolin-3-glucuronide of *Salvia officinalis*, while extraction time had no effect on mass fractions of total polyphenols and rosmarinic acid. Martins and da Conceicao (2015) showed that extraction temperature had a non-significant effect on rosmarinic acid and caffeic acid of *Apeiba tibourbou*, while extraction time had a significant effect on both rosmarinic acid and caffeic acid. Kuzmanović et al. (2015) reported that the temperature was the most significant factor affected phenolic compound content of corn silage. Our previous work investigated the effect of extraction temperature and time on centelloids content of *Centella asiatica*. We found that extraction temperature and time had a positive effect on centelloids content of *Centella asiatica* (Monton et al. 2018). However, the long extraction time perhaps led to decomposition of some plant chemical compounds such as phenolic compounds of *Centella asiatica* (Chew et al. 2011), polysaccharides from *Hovenia dulcis* peduncles (Liu et al. 2015), and polysaccharide from *Angelica sinensis* (Tian et al. 2017). The dual high temperature with long duration time led to degradation of some thermo-labile compounds (i.e., amygdalin of apricot-kernel and *Prunus tomentosa*) (Lv et al. 2005), polysaccharides of mulberry fruit (Chen et al. 2015), polysaccharides of *Tricholoma mongolicum* (Wang et al. 2015), and polysaccharides of *Astragalus cicer* (Shang et al. 2018). The above reports revealed that the optimal

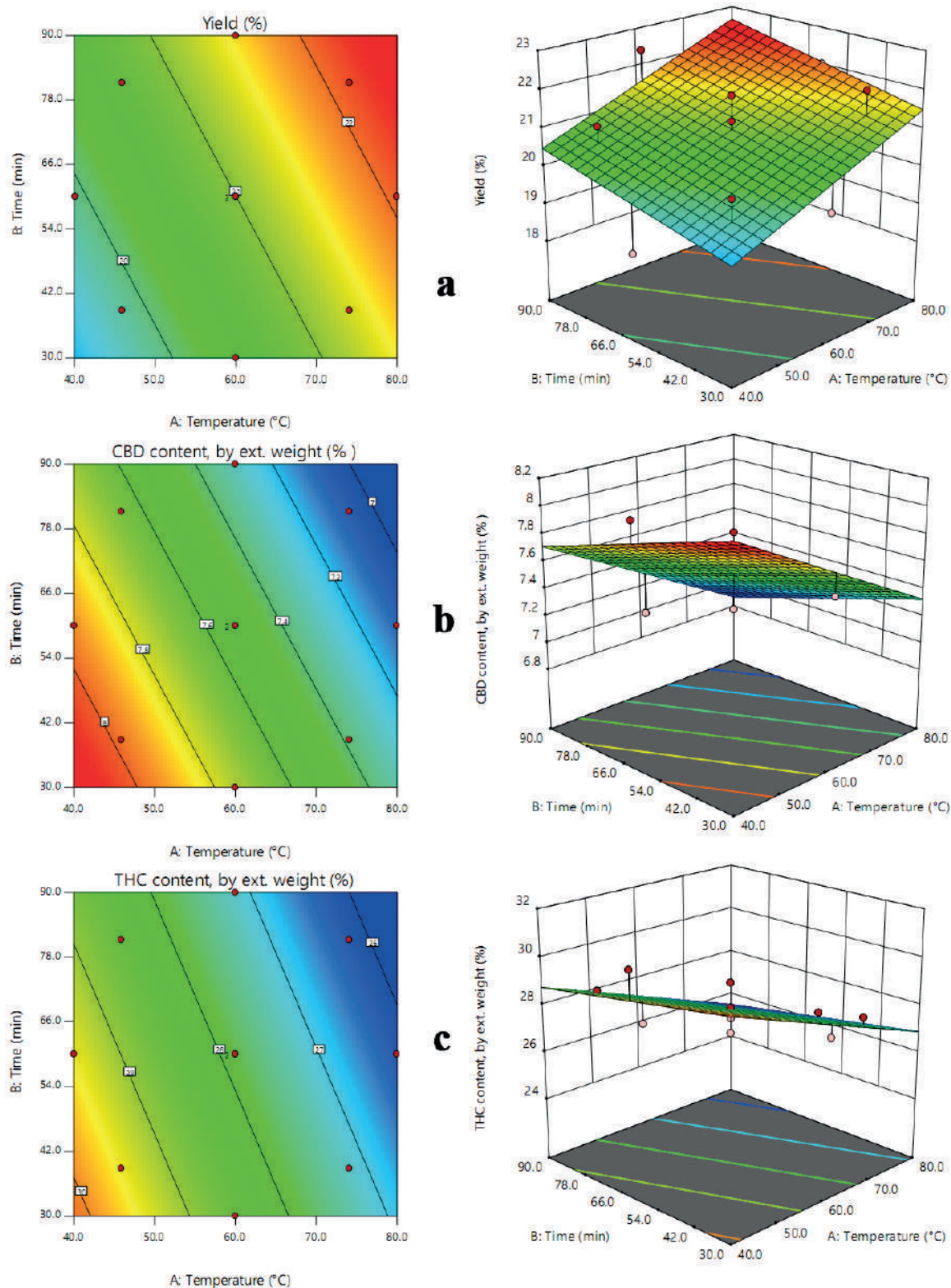


Figure 2 - Contour plots (left) and response surfaces (right) of model conditions of (a) yield, (b) CBD content, and (c) Δ^9 -THC content.

condition for the extraction of a specific plant should be optimized to ensure that the selected extraction condition provided the highest amount of active chemical compounds. Furthermore, it perhaps influences the biological and pharmacological effect of the plants.

Table II shows correlations between extraction yield, CBD content, and Δ^9 -THC content. The correlation between extraction yield vs. CBD content or extraction yield vs. Δ^9 -THC had a negative value with a significant manner. According to the correlation between CBD and Δ^9 -THC content, it had a high correlation with significant value. This result indicated that while CBD was extracted, Δ^9 -THC was extracted as well. This phenomenon perhaps related to the similar physicochemical properties of both compounds similarly affected extracted during the extraction process.

The optimal condition obtained from Design-Expert that provided the simultaneous high of CBD and Δ^9 -THC content was the temperature of 40 °C and duration time of 30 min. The desirability value of this condition was 0.946. The desirability plot (Fig. 3) showed that increasing temperature and duration time could decrease desirability value, indicating that temperature and duration time of the maceration had a negative effect on content of CBD and Δ^9 -THC in the cannabis extract, according to

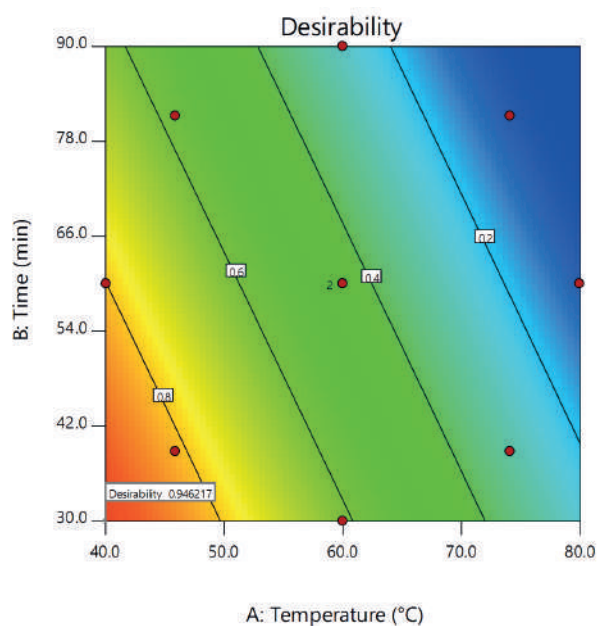


Figure 3 - Desirability plot.

Equations 2, 3, 5, and 6. This optimal condition was used to extract the cannabis to check the accuracy of the prediction by Design-Expert. Results showed that prediction by Design-Expert was accurate since the percent error was low (Table III).

CONCLUSIONS

The circumscribed central composite experimental design was applied to determine the effect of

TABLE II
Correlations between extraction yield, CBD content, and Δ^9 -THC content.

| Comparison | Pearson's correlation coefficient (R) | Coefficient of determination (R^2) | p-value |
|---|---------------------------------------|--|-----------|
| Yield vs. CBD content | -0.817 | 0.667 | 0.004* |
| Yield vs. Δ^9 -THC | -0.860 | 0.740 | 0.001* |
| CBD content vs. Δ^9 -THC content | 0.972 | 0.945 | < 0.0001* |

TABLE III
Predicted value, experimental value, and percent error of the prediction.

| Responses | Predicted value | Experimental value | %Error* |
|-----------------------------|-----------------|--------------------|---------|
| Yield (%) | 19.35 | 19.60±0.99 | 1.22 |
| CBD content (%) | 8.17 | 8.17±0.66 | 0.00 |
| Δ^9 -THC content (%) | 30.17 | 28.14±0.36 | -7.21 |

*%Error = (experimental value – predicted value)×100/experimental value.

maceration temperature and duration time on extraction yield, CBD content, and Δ^9 -THC content. The content of CBD and Δ^9 -THC had a negative correlation to the extraction yield. The high content of CBD and Δ^9 -THC were achieved when macerating using ethanol at the low heating temperature and short duration time. The optimal condition provided the simultaneous high of CBD and Δ^9 -THC content was a temperature of 40 °C and duration time of 30 min. The prediction was accurate and could be used as a guide for maceration of cannabis to obtain the extract containing a high content of CBD and Δ^9 -THC.

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AUTHOR CONTRIBUTIONS

CM is a project leader, designed the experiments, contributed to the experimental part, analyzed and interpreted the data, and drafted the manuscript. FM contributed to the isolation and purification of the standard compounds. SS contributed to the extraction part. TW, JS, and TS analyzed and interpreted the data. All authors have read and approved the final version of the manuscript.

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