Effect of Extraction Systems of Cannabinoid From Cannabis Sp on TLC-Densitometric Chromatogram Pattern

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ABSTRACT

Optimization studies of cannabinoid extraction method of Cannabis sp have been conducted. The aim of this study was to find a marijuana extraction method to identify cannabinoid compounds using TLC–Spectrophotodensitometry technique. This study covers the process of maceration and soxhlet extraction to extract cannabinoid content in marijuana flowers and seeds using methanol PE, and ether as a solvent, followed by a screening test using the TLC- Spectrophotodensitometry method. Confirmation test used Fast Blue B salt to visualize color of the spots. CBN, THC, and CBD can be separated by either using the stationary phase of Al-TLC S_I G_{60} F_{254} with a mobile phase of hexane - diethylether with the color visualization and Rf value after spraying with Fast Blue B Salt are the CBN will provide the purple-pink color with the HRF 59, THC will provide the purple color with the HRF 66, and the CBD will give orange color with the HRF 73 [1,2]. These results indicate that CBN, THC and CBD can be separated by either using the Al-TLC S_I G_{60} F_{254} system with a mobile phase of hexane-diethylether (80: 20, v / v). Differences in extraction methods affect the pattern of TLC-Densitometry chromatogram peak. This effect on the profiling of simplicia.

Keywords: extraction, cannabinoid, Al-TLC, Spectrophotodensitometry

INTRODUCTION

Flowers and seeds of marijuana (Cannabis sp) has the main content of cannabinoid compounds, which are known to have four main cannabinoid, the CBN (cannabinol), CBG (cannabigerol), CBD (cannabidiol) and THC (Δ9-Tetrahidrocannabinol). Cannabinoid content between species are different each other. Cannabinoid

biosynthesis occurs in gland pockets scattered on the surface of the marijuana plants and this biosynthesis is strongly influenced by climate and environment [3].

Biosynthesis of secondary metabolites will be influenced by the conditions of these plants. So the content of the resulting metabolites could be different. Differences in climate and special varieties affect the metabolites content of these Cannabis sp [3]. Based on these differences, it can be used as a basis for profiling analyzing the chemical content of marijuana.

Extraction method and solvent of secondary metabolites of Cannabis sp also determine differences in components of metabolites to be extracted [4,5]. Therefore it is necessary to optimize of extraction methods of cannabinoids from marijuana flowers and seeds. In this study, the secondary metabolites extracted be separated and detected by using TLC-Spectrophotodensitometry, which has a high specificity and can be used for the sample simultaneously. To produce a good separation, required the selection of chromatographic systems which include both stationary and mobile phase. Chromatogram character of components that have been separated then be analyzed, include color, spectrum, Rf and AUC values. So, cannabinoid compounds that contained in cannabis flowers and seeds can be identified. By the optimum extraction method and chromatographic system, it is expected to be obtained great randemen and the best separation, which can support the implementation of drug profiling analysis of Cannabis sp.

MATERIALS AND METHODS

Materials

Chemicals used in this study were methanol, PE, ether (technical grade), toluene p.a, hexane p.a, diethylether p.a (Merck Germany), Fast Blue B salt, distilled water, Al-TLC Si Gel 60 F254 plates (Merck, Darmstadt, Germany), and HPTLC Si Gel 60 F254 plates (Merck, Darmstadt, Germany). The samples used in this study is cannabis flowers and seeds obtained from the forensic chemistry laboratory Police Headquarters Denpasar Branch.

Equipment

The tools used include glass tools, analytical scales, a set of soxhlet,

rotary vacuum evaporator, Eppendorf tubes, chamber vessel size 10x10 cm (Camag, Muttenz, Switzerland), oven (Memmert), nanomat IV, TLC-Scanner 3 (Camag -Muttenz-witzerland), centrifugator (centrifuge Micro-Clements), and the sprayer (TLC sprayer Camag, Muttenz, Switzerland).

Methods

Extraction Process

Maceration

About 500 mg of dried powder samples were added with 15 mL of methanol, PE, or ether solvent, macerated for 24 hours. After 24 hours of the extract was filtered and evaporated in the evaporator at 40 ^{0}C for methanol, 50 ^{0}C for PE, and 30 ^{0}C for ether until thick extract formed. After that add 1 mL of toluene, and then pipetted and put into eppendorf tubes, centrifugate at 3500 rpm for 15 minutes. The upper phase were collected and the total volume were recorded.

Soxhlet extraction process

Soxhlet extraction was performed on marijuana samples by using methanol and PE solvent at 40 ^{0}C for 5-6 round of solvent flow. The extract obtained was evaporated at 40^{0}C for methanol, 50 ^{0}C for PE until thick extract formed. After that add 1 mL of toluene, and then pipetted and put into eppendorf tubes, centrifugate at 3500 rpm for 15 minutes. The upper phase were collected and the total volume were recorded.

Screening Test

Identification process carried out by TLC / HPTLC Spectrophotodensitometry method. Identification of CBD, CBN, and THC carried out by using three solvent system: hexane-diethyl ether (80: 20, v / v), toluene, toluene-chloroform-methanol (100: 10: 1, v / v) with two stationary phases, HPTLC SI G 60 F254 and Al-TLC SI G 60 F254. Each 3 ml sample spotted on the plate, and then eluted by mobile phase. Plate which had been eluted, then scanned with a TLC-scanner at 210 nm. The spectrum of each peak of chromatogram were scanned in situ at wavelength 190-400 nm.

Confirmation Test

The assay is performed using a solution of Fast Blue B salt.Scanned plate then sprayed with Fast Blue B Salt. These reagents will provide the

color violet for CBN, Orange-Red to purple for the CBD and THC. Calculation of the HRF, the AUC and comparison with library spectra of analytes is accomplished using the WinCATS programe. All results obtained were compared with the library so it can be ascertained that the analytes are the CBN, CBD and THC.

RESULTS AND DISCUSSION

Extraction process

Methanol, PE, and ether was chosen as solvents because these solvents can dissolve the compound CBN, THC and CBD in cannabis flowers and seeds. Ether not be used for soxhlet extraction because its boiling point is very low, at 30 ^{0}C and is highly flameable and evaporable when heated, so that when used as extracting solvent would pose considerable danger.

Screening test

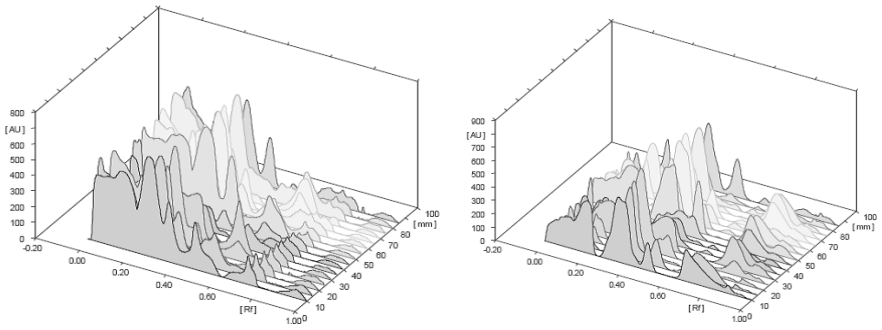
At this stage HPTLC SI G60 and Al-TLC SI F254 G60 F254 plate spotted by 3 µl sample were scanned at 210 nm to obtain the chromatogram pattern of entire line in each sample.

Confirmation test

Plate that has been scanned then sprayed with Fast Blue B salt so color of CBN, THC and CBD can be visualized and Rf values can be counted. Rf values and color vizualisation of each spot are showed in table 2.

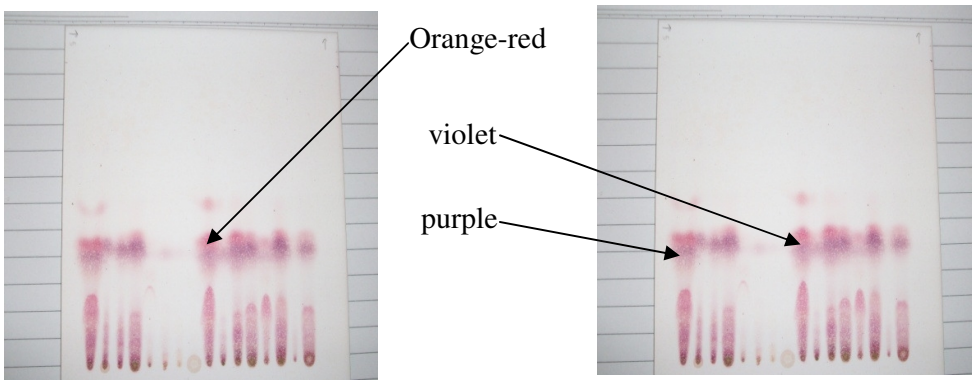
Table 1. Extract Proceed

Method	Sample	Solvent	Extract Volume
Maseration	Flower	Methanol PE Ether	1.3 mL 1.14 mL 1 mL
Maseration	Seed	Methanol PE Ether	1.52 mL 1 mL 1.72 mL
Soxhlet Extraction	Flower	PE Methanol	1.57 mL 1 mL
Soxhlet Extraction	Seed	PE Methanol	1 mL 1 mL



(a) (b)

Figure 1. Chromatogram pattern of each sample in hexan-diethyllether (80:20 v/v) mobile phase, Al-TLC SI G_{60} F_{254} plate (a) dan HPTLC SI G_{60} F_{254} plate (b).



(a) (b)

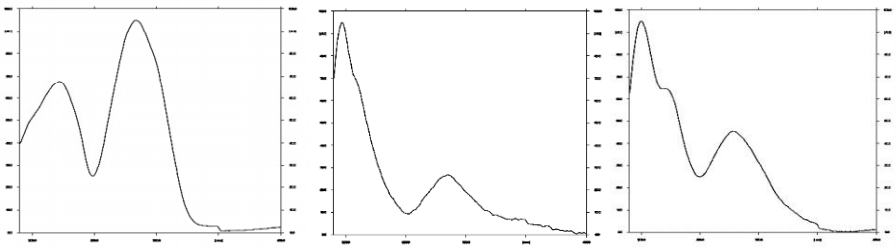
Figure 2. Color vizualisation of each spot on hexane-diethylether (80:20 v/v) mobile phase after sprayed with fast blue B salt.
(a) Plat Al-TLC SI G $_{60}$ F_{254} plate, (b) Plat HPTLC SI G $_{60}$ F_{254} plate

Table 2. Rf value and color visualization on AL-TLC S1 G_{60} F_{254} plate and hexane-diethylether (80 : 20, v/v) mobile phase.

Sample	Spot	Color	Rf
Flower (PE)	1	Purple	0.05
Soxhlet	2	Violet	0.16
	3	Violet	0.38
	4	Violet	0.51
Flower	1	Purple	0.11
(Methanol)	2	Purple	0.29
Maseration	3	Violet	0.36

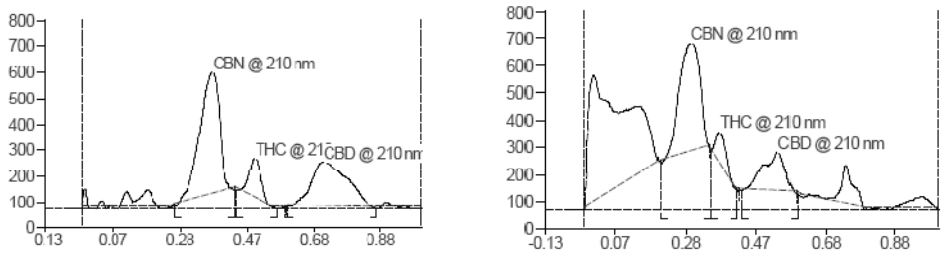
	4	Violet	0.50
Flower (PE)	1	Purple	0.09
Maseration	2	Purple	0.29
	3	Orange-red	0.40
Flower (Ether)	1	Purple	0.09
Maseration	2	Purple	0.29
	3	Orange-red	0.41
Seed (Methanol)	1	Violet	0.06
Soxhlet	2	Orange-red	0.18
	3	Purple	0.35
	4	Violet	0.65
Seed (Methanol)	1	Violet	0.08
Maseration	2	Purple	0.29
	3	Orange-red	0.41
Seed (PE)	1	Orange-red	0.06
Maseration	2	Violet	0.29
Seed (Ether)	1	Orange-red	0.23
Maseration			

Attachment between one and another spot can cause a buildup peak of compounds, making it hard to identify compounds CBN, THC and CBD. To overcome this, sample spectrum can be compared with spectra library by using WinCATS program and also compare the Rf value of each spot with Rf value in reference books. So it can be determined the position of these compounds on the chromatogram. Spectrum of CBN, THC, and CBD can be seen in Figure 3. After tagging, it is known where the position of CBN, THC and CBD in each chromatogram, as shown in Figure 4.



(a) (b) (c)

Figure 3. Insitu spectrum of CBN (a), THC (b), dan CBD (c).



(a) (b)

Figure 4. Chromatogram of flower sample (maseration with methanol) in hexane-diethylether (80:20 v/v) mobile phase, HPTLC SI G_{60} F_{254} plate (a) dan Al-TLC SI G_{60} F_{254} plate(b).

Mobile Phase Optimation

The resolution (Rs) of separation of CBN, THC and CBD on each stationary phase and mobile phase can be calculated based on the mobile phase chromatograms.

Table 3. Rs vlue of cannabinoid in hexane-diethylether (80:20, v/v) mobile phase.

Sample	Compound	Rs			
		HPTLC		TLC	
		THC	CBD	THC	CBD
Flower (PE) Soxhlet	CBN	1.00	1.50	1.45	-
	THC	-	1.12	-	-
Flower (Methanol) Maseration	CBN	0.83	1.43	0.81	2.27
	THC	-	1.08	-	2
Flower (PE) Maseration	CBN	0.81	2.07	1.75	2.26
	THC	-	1.73	-	1.85
Seed (PE) Soxhlet	CBN	0.96	6.4	3.27	2.55
	THC	-	2	-	1.18
Seed	CBN	0.81	3.63	0.8	3.75

(Methanol) Maseration					
	THC	-	1.3	-	1.83
Seed (PE) Maseration	CBN	1.04	4.14	2.37	3.54
	THC	-	1.62	-	1.33

Two compounds are well separated if they have Rs ≥ 1 [6]. Based on the data obtained, the best separation is shown by hexane-diethylether (80: 20, v / v) mobile phase (System TAH) with Al-TLC SI G60 F254 plate where the system separates compound with an average value of Rs ≥ 1 and also able to separates the CBD, CBN, and THC compounds from marijuana flowers and seeds as well. It can also be seen from the emergence of CBN, THC and CBD peak in the absence of the buildup peak of compounds. These results are consistent with research conducted by Galand, et al in 2004, which states that the stationary phase of Al-TLC SI G60 F254 with hexane-diethylether mobile phase is the best method used for qualitative analysis of the CBD, THC and CBN [2].

Extracting Solvent Optimization

Assessment of the best extracting solvent can be seen from the number of Cannabidiol (CBD), Cannabinol (CBN), and Δ9-Tetrahidrokannabinol (THC) which can be extracted based on the total AUC of each extraction method. From the data of percentage of AUC values of cannabis seeds on AL-TLC Si F254 G60 plate (Table 4) there is little difference in the results with previous data. The data shows that the highest total AUC value obtain by soxhlet method with PE solvent that is equal to 17558.3 and the next position is obtained by maceration method with methanol solvent that is equal to 14051.5.

AUC values on method maceration with methanol solvent looks bigger than on soxhlet method with methanol solvent. It is slightly different from the statements of Kovar and Auterhoof which says cannabis is better extracted with soxhlet tool [5]. It can be expected that evaporation the content of cannabinoid compounds can occur during the extraction process using soxhlet method. Data from Table

4, shows that the total AUC of soxhlet method using PE solvent is the biggest, but the percentage AUC of THC compound is very small (0.6%). While the maceration method using methanol solvent, the percentage range of AUC values of CBN, THC, and CBD are 18.8% : 54.3% : 26.8%.

Tabel 4. Persentase AUC CBN, THC, CBD pada sampel biji ganja dengan fase gerak heksan-dietileter (80:20, v/v). Fase diam Al-TLC SIG_{60} F_{254}.

Sample	%AUC			AUC TOTAL
	CBN	THC	CBD	
Seed (PE) Soxhlet	25.1	0.6	74.3	17558.3
Seed (Methanol) Soxhlet	83.8	8.7	7.5	11522.7
Seed (Methanol) Maceration	18.8	54.3	26.8	14051.5
Seed (PE) Maceration	50.0	15.5	34.4	2881.6
Seed (Ether) Maceration	9.0	51.8	39.2	7461.2

Based on that data, it can be said that maceration method using methanol solvent is the optimum extraction method to extract CBN, THC and CBD compounds from marijuana seeds, especially for analysis of drug profiling purpose. Selection of the optimum extraction method is based on the smallest percentage AUC range between one to another compound and AUC value of THC is quite high.

CONCLUSION

From result of this research, it can conclude that :

A. The optimum extraction method of CBn, THC and CBD is maceration method using methanol solvent wich rasio level obtained 1 : 3 :1.

B. Hexane-diethylether (80:20 v/v) mobile phase (TAH system) with Al-TLC Si G60 F254 stationary phase is the optimum system to separate CBN, THC and CBD compounds from marijuana flowers and seeds.

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