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Comparison of the Two Common Solvents for THC and CBD Extractions

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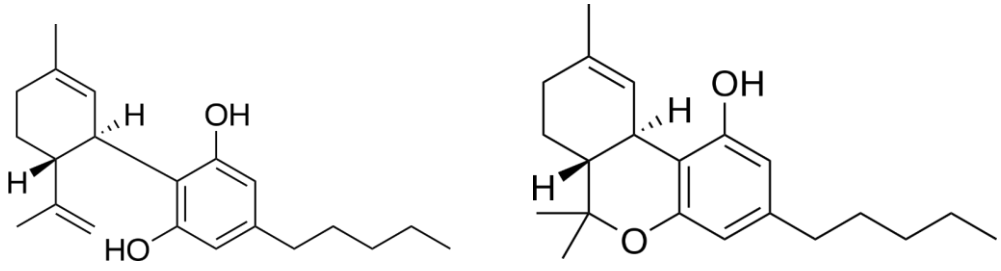
Abstract **-** Cannabis extract (*Cannabis sativa L.*) has been widely used for both medical and recreational purposes. The ability of cannabis to exert effects on health varies depending on different amounts of the active compound, cannabinoids. The important ingredients of interest are namely Delta-9-tetrahydro cannabinoids (THC) and Cannabidiol (CBD). It is known that the common solvent used for the extraction process is ethanol, among a variety of organic solvents. Accounted for the low polarity of those interest cannabinoids, other common solvents of higher carbon chain such as isopropanol are subjected to study for comparing the extracted concentrations of THC and CBD at the same condition with ethanol. The experiment was conducted using the same amount of dried cannabis leave and flowers in two solvents; ethanol and isopropanol. The filtrate was dried under vacuum using Rotary Evaporator and subjected to the Liquid-Chromatography techniques. Fractions were collected and tested with the thin layer chromatography technique (TLC) with respect to the standard solution. Liquid Chromatography was applied to separate the constituents, followed by the high-performance chromatography technique (HPLC) for the quantification of THC and CBD. The results showed that CBD, which is higher polarity, was obtained in the ethanol extract more than that of isopropanol. Whist, isopropanol solvent provided the higher amount of THC attributed to the more compatibility between lower polarities of substances. Therefore, it is recommended that the selection of solvent depends on the main target of the ingredients required in the extract.

Keywords**:** Cannabis extraction, Delta-9-tetrahydro cannabinoids, Cannabidiol, ethanol extract, isopropanol extract

1. Introduction

Cannabis (Cannabis sativa L.) or marijuana belongs to the family of the Cannabaceae [1]. It contains a wide variety of chemicals, with approximately 500 compounds have been identified [1, 2]. The major chemical cannabinoid constituents are including delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) whereas other cannabinoids found are cannabinol (CBN), cannabigerol (CBG), and cannabichromene (CBC) [2, 3], which are the medicinally use for glaucoma [4], chronic neuropathic pain [5], schizophrenia [6], intestinal dysfunction [7], rheumatoid arthritis [8] and others.The psychotropic effects of cannabis are mediated by THC. This study had paid the interest to THC and CBD, which are the bi- and tricyclic compounds containing twenty-one atoms of carbon, thirty of hydrogen, and two of oxygen as shown in Figure 1.



(a) (b)

Fig. 1: The structure of CBD (a) and THC (b)

Many previous research works had formulated new medicinal products such as the Oromucosal spray from cannabis extract, cannabis balm, cannabis oil, and the cannabis wafer that provides a simpler solution than other cannabis products [9]. The extraction processes were commonly using liquid extraction with organic solvents such as hexane, chloroform, and petroleum ether. Among these choices, ethanol (EtOH) has been the most common solvent used for many purposes. Considering one more carbon in the branched structure of isopropyl alcohol (iPrOH), it may provide another suitable solvent for a specific purpose directed to the important ingredients mentioned above. For this reason, this study aims to compare the efficacy of the two organic solvents for suiting the ingredients of cannabis; THC and CBD.

2. Objective

The purpose of this study is to compare the capability of those two common solvents toward the amount of CBD and THC ingredients in the extracts.

3. Methodology

The dried cannabis samples (Cannabis sativa L., Cannabaceae) were supplied from illegal narcotic drugs in Thailand. THC and CBD reference standards were supported by The Herbal Medicinal Products Research and Development Center, Rangsit University. AR grade EtOHand iPrOH was purchased from Aldrich. All other chemicals were used as received without further purification. Thin-layer chromatography was developed using the eluant mixture with the ratio of 1:1 ethyl acetate: hexane. A spray agent was used to clarify the pattern of the separation.

The quantitative analysis of THC and CBD was succeeded using a reverse-phase of high-performance liquid chromatography (HPLC) method and diode array at 222 nm. The mobile phase is a mixture of 90:10 of methanol and water with a flow rate of 1.0 ml/min under gravity force through a reverse-phase of Zorbax C-18 column 4.6 mm × 150 mm, 5.0 micrometers [10]. All experiments were carried out at 30 ± 0.5 ^{o}C. The peak identification was performed by comparing the retention times of the samples with those of the standard solutions. Peak areas of fractions at the specific retention time are calculated using the LC solution software and reflected the total concentration of THC and CBD of each solvent. The suitable extractant for important ingredients was then can be concluded.

4. Result and Discussion

The double replication crude extracts of EtOHand iPrOH were yielded after the evaporation process of the filtrates. Both extracts have similar physical appearances with browny green color as shown in Figure 2.

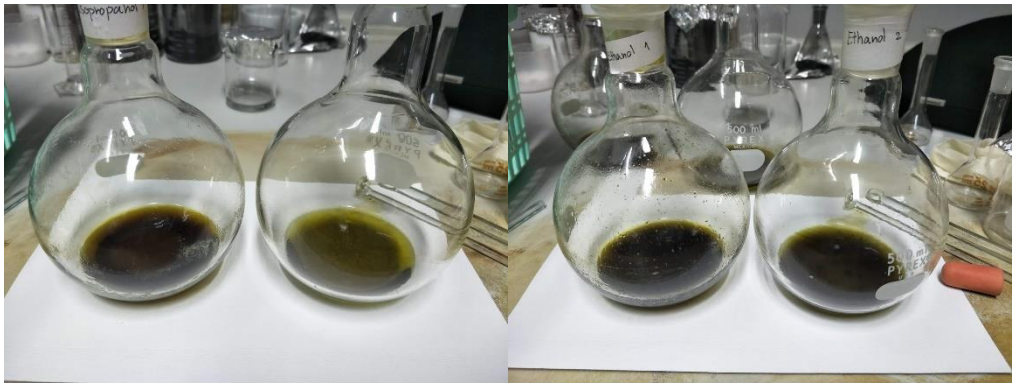


Fig. 2: The crude extracts of cannabis using EtOH (a) and iPrOH (b) as the extractants.

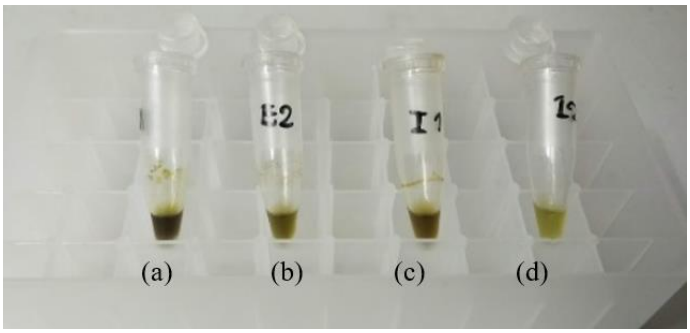


Fig. 3: Diluted extract of the sample for TLC test

A portion of extracts was diluted (Figure 3) and subjected to a TLC aluminum sheet, silica gel 60, and GF254. The chromatogram was compared with the standard solutions of CBD, CBN, and THC, respectively. The spray agent, which is a mixture of anisaldehyde, acetic acid, methanol, and sulphuric acid, enhanced the appearance of the pigments. The TCL results of extracts, duplicated of the EtOH extracts (E1 and E2) and iPrOH extracts (I1 and I2), are shown in Figure 4.

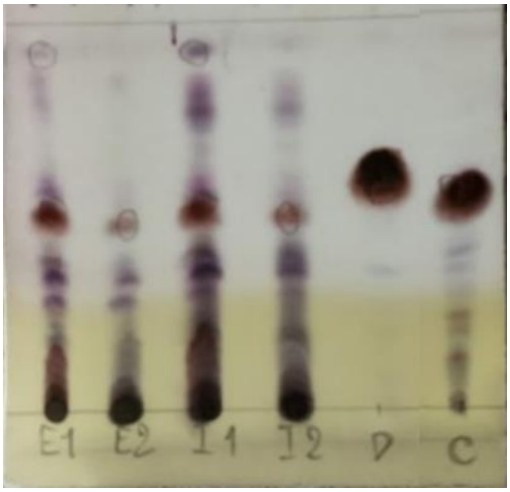


Fig. 4: TLC test of the sample compared with the standards of CBD (D), and THC (C)

Taking into account the same RF, both extracts from EtOH and iPrOH probably contained the important ingredients of CBD and THC. It should be mentioned here that the extracts are composed of a variety of substances, for example, turpine and hundreds of cannabinoids [11]. Those replications of the samples were subjected to the column chromatography filled with silica pore size 60 micron (Figure 5). The mixture of 1:1 of dichloromethane/hexane was used as an eluant. Besides, it should be noted that the separation can be noticed by the color appearance in the column. The beginning fractions performed the browny color of pigments, followed by the brownish-green and pale greeny, respectively. The collected fractions of 15 mL were labeled as 1-10 and tested with the thin layer chromatography for recruiting the fraction composing of THC and CBD. Figure 6 shows the chromatogram of the fractions collected from both solvents compared with the standard of CBD and THC.

It was found that both extractants provided rather the same results. The fractions 1-6 showed the existence of the cannabinoids suggested by the same rate of flow (RF) as the standards. Without further purification, all fractions were subjected to quantified using High-performance liquid chromatography (HPLC). Based on the retention time and peak areas, the samples could be roughly determined for the amount of CBD and THC. The determination of THC and CBD was succeeded using the isocratic elution reverse-phase HPLC. The mobile phase was the mixture of methanol 90%(v/v) in water

[2, 3]. This condition obtained a good separation and gave a clear sharp peak and this mobile phase was developed and proved to be a good linear relationship of CBD and THC as shown in Figure 7. The retention times showed the peak area at 4.643 min and 12.328 min, respectively. The different retention time was attributed to the difference of the substance polarity. CBD bears with higher polarity than THC with responding to the hydroxy group of CBD.

Fig. 5: Column chromatography of the crude extracts

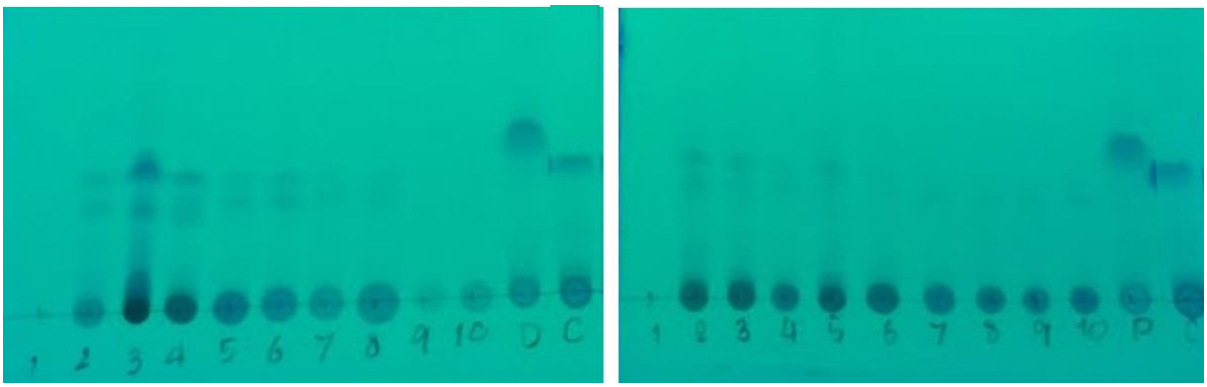


Fig. 6: TLC test of fractions 1-10 collected from column chromatography compared with the standard of CBD (D), CBN (N), and THC (C): (a) EtOH extract (b) iPrOH extract.

It was found that both extractants provided rather the same results. The fractions 1-6 showed the existence of the cannabinoids suggested by the same rate of flow (R_F) as the standards. Without further purification, all fractions were subjected to quantified using High-performance liquid chromatography (HPLC). Based on the retention time and peak areas, the samples could be roughly determined for the amount of CBD and THC. The determination of THC and CBD was succeeded using the isocratic elution reverse-phase HPLC. The mobile phase was the mixture of methanol 90%(v/v) in water [2, 3]. This condition obtained a good separation and gave a clear sharp peak and this mobile phase was developed and proved to be a good linear relationship of CBD and THC as shown in Figure 7. The retention times showed the peak area at 4.643 min and 12.328 min, respectively. The different retention time was attributed to the difference of the substance polarity. CBD bears with higher polarity than THC with responding to the hydroxy group of CBD.

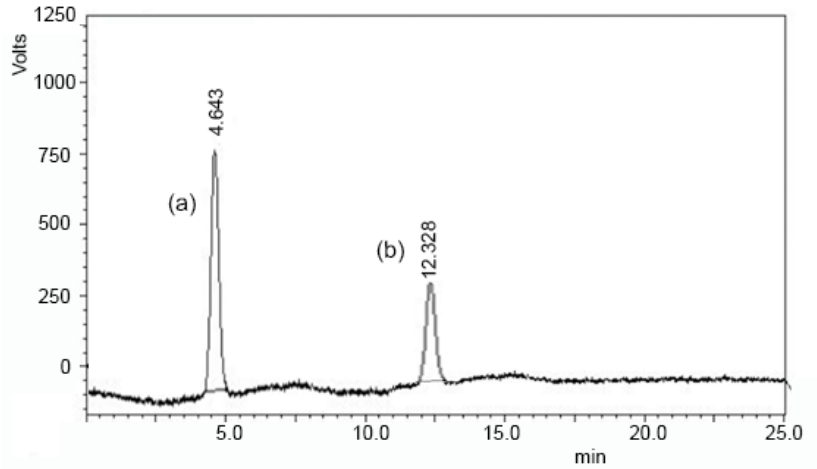


Fig. 7: Chromatogram of a standard solution of (a) CBD and (b) THC at the concentration of 100 µg/ml

Good linearity of standards was obtained by peak areas of the six different standard solutions [10]. Crude extracts were run through the Liquid chromatography and 10 fractions were collected and subjected to HPLC. The peak areas data at RT in the range of 4.63-4.67 and 12.19-12.32 are defined as CBD and THC, respectively. Table 1 shows the peak areas for fractions extracted by EtOH and iPrOH. The comparison between peak areas reflects the capacity of the extraction solvent. The higher peak area for EtOH extract suggests the better capacity of extraction for CBD of ethanol. On the other hand, the higher peak area at THC retention time reveals the better capacity extraction for THC of isopropanol.

Table 1: Peak area of EtOH and iPrOH extracts defined as CBD concentration for each fraction.

Fractions	**Peak area for CBD contents** (R_T = 4.63-4.67)		**Peak area for THC contents** (R_T = 12.19-12.32)	
	EtOH	iPrOH	EtOH	iPrOH
1	n.d.	n.d.	12967	17327
2	858902	1797958	93289	192832
3	1801099	448298	59898	19374
4	224633	51202	28347	19561
5	35732	57473	27685	22257
6	45477	26889	31231	13263
7	39142	4810	37413	20881
8	23908	7174	28567	13842
9	1705	4851	n.d.	18429
10	n.d.	5661	n.d.	20044
Total peak area	**3030598**	**2404316**	**306430**	**340483**

Quantification of the concentrations of CBD and THC in each fraction (fractions 1-10) was conducted and graphical reported in Figure 8.

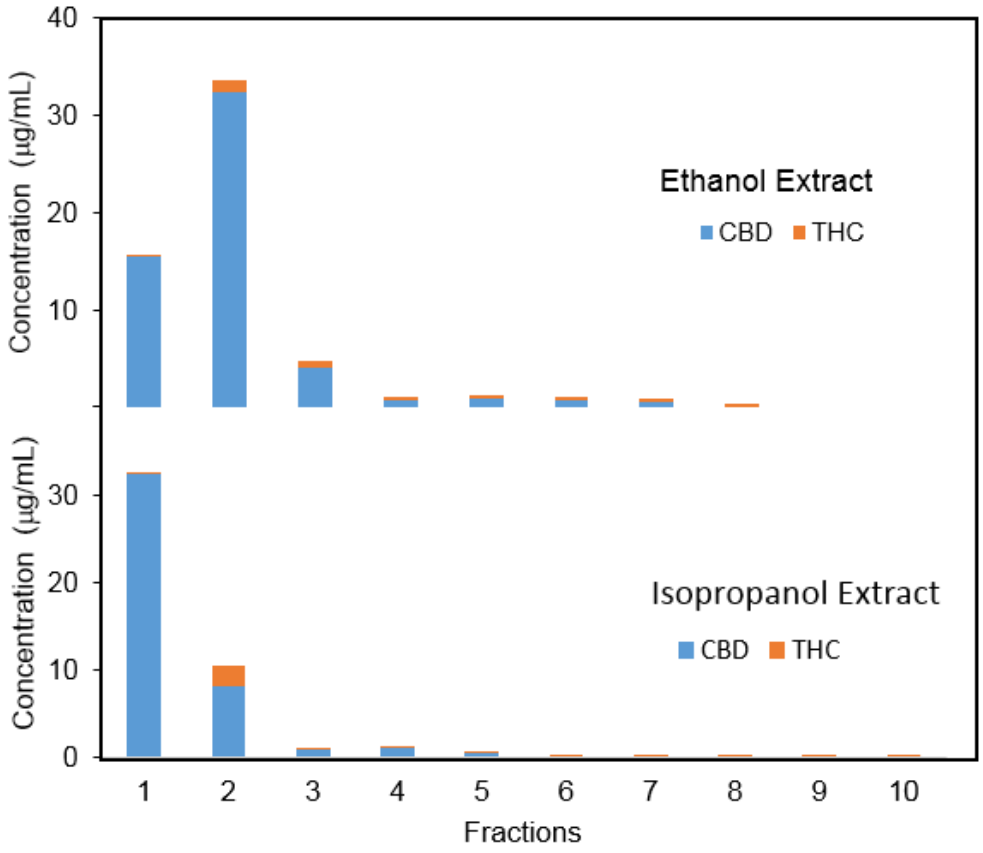


Fig. 8: Contents of cannabinoids (■ = CBD and ■ = THC) in fractions

The stacked bar plot depicted a huge difference between the concentration of CBD and THC in the first group, particularly for the fractions 1-3. These results suggested that CBD contents in the studied cannabis were much higher than that of THC. The earlier fractions of 1-3 also provide the important ingredients over the rest of the fractions. These results were in accordance with that of Fameera Madaka et al [3] who reported the extracted ratio content of CBD/THC is 4.5 times in her study.

The summation of the peak areas for CBD and THC contents from all fractions was elucidated to reflect the solvent ability between the EtOH and iPrOH extractants in which the more peak areas provided the higher content of the samples. The calculation of gram contents of CBD and THC in the sample was accomplished in the vicinity of the starting solution prepared by soaking 10 g of dried cannabis sample (leaf and flower) in 450 mL of the solvents. Table 2 shows tabulated yields in µg unit and ranges of R_T obtained for the products of CBD and THC.

Table 2: Contents in EtOH and iPrOH extracts

Ingredient	R_T of EtOH extract	R_T of iPrOH extract	Ingredient in EtOH extract (µg)*	Ingredient in iPrOH extract (µg) *
CBD	4.63-4.67	4.64 - 4.66	818.26	649.17
THC	12.19-12.32	12.26-12.31	57.49	64.41

**10 gms of dried cannabis in 450 mL of solvents*

From Table 2, a higher amount of CBD could be obtained by EtOH extractant rather than that of iPrOH extractant whereas the iPrOH was seemed to be a better solvent for THC products. This result can be attributed to the lower polarity of the THC solubilized in the lower polarity solvent of iPrOH and vice versa of EtOH and CBD. However, to produce medical

products, CBD is paid more attractive ingredient than THC due to its protective effect against the negative psychological effects related to THC including the antagonizing of the adverse effects from THC. Therefore, EtOH can be more suitable solvent not only recruiting the high yield of CBD but also the lower price of that common solvent.

4. Conclusion

The comparison of the capability of solvents between ethanol and isopropanol as the extractant for CBD and THC ingredients in dried cannabis samples was done. The liquid extraction experimental was applied under a suitable condition for the local market of cannabis oil production. The amount of CBD and THC in the extracts yielded from the liquid extraction process were calculated based on the peak areas provided from the HPLC chromatograms referenced by the standard solution. It was found that both solvents were comparatively good extracting solvents toward the high contents of CBD and THC. This study also revealed under the two replicates that a higher yield of CBD can be obtained from ethanol extract whilst THC could be extracted in higher contents under the vicinity of isopropanol. Therefore, the development of medical products with a high ratio of CBD/THC should be preferable via ethanol extract, which is the most common cheap solvent in all dimensions of usage.

Acknowledgments

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