EXTRACTION OF CANNABINOIDS USING VEGETABLE OILS AND ITS UVA-PHOTOPROTECTIVE EFFECT ON HUMAN SKIN KERATINOCYTES

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การสกัดสารแคนนาบินอยด์ด้วยน้ำมันพืชและฤทธิ์ปกป้องแสงยูวีเอต่อเซลล์คีราติโนไซต์ผิวหนังมนุษย์

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เนอร์ อัลฟาน มูฮัมหมัด เซน : การสกัดสารแคนนาบินอยด์ด้วยน้ำมันพืชและฤทธิ์ปกป้องแสงยูวีเอต่อเซลล์คีราติโนไซต์ผิวหนังมนุษย์. (EXTRACTION OF CANNABINOIDS USING VEGETABLE OILS AND ITS UVA-PHOTOPROTECTIVE EFFECT ON HUMAN SKIN KERATINOCYTES) อ.ที่ปรึกษาหลัก : รศ. ภญ ดร.สรกนก วิมลมั่งคั่ง

ปัจจุบันน้ำมันพืชได้รับความสนใจในฐานะตัวทำละลายทางเลือก ความสามารถในการละลายสารออกฤทธิ์ต่าง ๆ จากพืชได้รับการพิสูจน์โดยการศึกษาก่อนหน้านี้หลายครั้ง นอกจากนี้ ประโยชน์อื่น ๆ ของการใช้น้ำมันพืชเป็นตัวทำละลาย คือ ราคาจับต้องได้ ไม่เป็นพิษ และเป็นมิตรกับสิ่งแวดล้อม กระบวนการสกัดเป็นหนึ่งในปัจจัยสำคัญที่ส่งผลต่อคุณภาพของกัญชา เอทานอลยังคงเป็นตัวทำละลายที่ดีที่สุดสำหรับการสกัดกัญชาในแง่ของการให้ปริมาณสิ่งสกัดจากการผลิตเมื่อเทียบกับตัวทำละลายอินทรีย์อื่นที่ใช้ในการศึกษานี้ รวมถึงน้ำมันที่ไม่สามารถคำนวณผลผลิตได้เนื่องจากน้ำมันพืชไม่สามารถระเหยได้ ลายพิมพ์นิ้วมือ HPTLC เผยให้เห็นว่าสารสกัดน้ำมันกัญชาที่สกัดโดยใช้น้ำมันพืชหลากหลายชนิด ได้แก่ น้ำมันมะพร้าวบริสุทธิ์ (VCO) น้ำมันมะกอก (OO) น้ำมันเมล็ดงาขี้ม่อน (PSO) น้ำมันถั่วดาวอินคา (SIO) น้ำมันรำข้าว (RBO) และน้ำมันเมล็ดงา (SSO) แสดงโพรไฟล์ของสารกลุ่มแคนนาบินอยด์ที่คล้ายกันกับสารสกัดตัวทำละลายอินทรีย์ โดยระบุได้จากการปรากฏของ CBG, CBN, THC และ CBD อิทธิพลของกระบวนการให้ความร้อนก่อนการสกัดได้รับการทดสอบ พบว่าสารแคนนาบินอยด์ทั้งในรูปแบบที่เป็นกรดและเป็นกลางนั้นถูกตรวจพบแม้กระทั่งดอกกัญชาจะถูกให้ความร้อนก่อนทำการสกัดแล้วก็ตาม สิ่งนี้บ่งชี้ว่ากระบวนการให้ความร้อนก่อนการสกัดไม่มีประสิทธิภาพเพียงพอในการทำให้ปฏิกิริยาดีคาร์บอกซิเลชันสมบูรณ์ การสกัดกัญชาที่ได้จากสำนักงานป้องกันและปราบปรามยาเสพติดประเทศไทย โดยกลุ่มตัวทำละลายที่ระเหยได้มีแนวโน้มให้ผลผลิตเนื้อสาร CBD สูงกว่า THC ในทางตรงกันข้ามตัวทำละลายแบบคาร์บอนไดออกไซด์วิกฤตยิ่งยวด ทำให้ได้เนื้อสาร THC ที่สูงกว่า CBD และให้ปริมาณสูงสุดของทั้ง THC และ CBD เมื่อเทียบกับตัวทำละลายอื่นๆ น้ำมันพืชให้ปริมาณต่ำสุดของทั้ง CBD (0.01-0.02% w/w) และ THC (0.02-0.03%w/w) สำหรับการทดสอบฤทธิ์ต้านอนุมูลอิสระนั้น พบว่า CBG, CBN, THC, CBD และสารประกอบที่ไม่ทราบชื่ออื่น ๆ มีฤทธิ์นี้ภายในกลุ่มของตัวทำละลายอินทรีย์และกลุ่มของน้ำมันพืช สารสกัดคาร์บอนไดออกไซด์วิกฤตยิ่งยวดที่ผ่านกระบวนการวินเทอร์ไรเซชันแล้ว (W-$ScCO_2$) และสารสกัด RBO แสดงฤทธิ์ที่แรงที่สุดด้วยค่า IC50 ที่ 0.03 ± 0.006 มก./มล. และ 3.35 ± 0.120 มก./มล. ตามลำดับ นอกจากนี้สารสกัดเอทานอล, สารสกัด W-$ScCO_2$ และสารสกัด VCO ได้ถูกนำไปทดสอบฤทธิ์ป้องกันแสง UVA ต่อเซลล์ HaCat พบว่าสารสกัด W-$ScCO_2$ ปกป้องเซลล์ที่ฉายรังสี UVA ได้อย่างมีนัยสำคัญ ในขณะที่สารสกัด VCO รวมถึง CBD และ THC บริสุทธิ์มีผลเพียงเล็กน้อย

คำสำคัญ: กัญชา; น้ำมันมะพร้าวบริสุทธิ์; แคนนาบิไดออล; เวชสำอาง

สาขาวิชา	เภสัชศาสตร์และเทคโนโลยี	ลายมือชื่อนิสิต .....
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Nur Alfan Muhammad Zen : EXTRACTION OF CANNABINOIDS USING VEGETABLE OILS AND ITS UVA-PHOTOPROTECTIVE EFFECT ON HUMAN SKIN KERATINOCYTES. Advisor: Assoc. Prof. SORNKANOK VIMOLMANGKANG, Ph.D.

Nowadays, vegetable oil has attracted attention in its role as an alternative solvent. Its ability to dissolve various active compounds from plants has been proven by several previous studies. Moreover, other benefits of the use of vegetable oil as a solvent are affordable, non-toxic, and eco-friendly. The extraction process is one of the crucial factors that affect the quality of cannabis. Ethanol is still consistently the best solvent for cannabis extraction in terms of yield production compared to other organic solvents used in this study including oil whose yield cannot be calculated since vegetable oil cannot be evaporated. HPTLC fingerprint revealed that the cannabis oil extracts using various vegetable oils including virgin coconut oil (VCO), olive oil (OO), perilla seed oil (PSO), sacha inchi oil (SIO), rice bran oil (RBO), and sesame seed oil (SSO) showed a similar cannabinoid profile with the organic solvent extracts which were indicated by the presence of CBG, CBN, THC, and CBD. The influence of the heating process before extraction was tested. It was found that both acid and neutral forms of cannabinoids were detected even the cannabis flowers were heated before extraction. This indicated that the heating process before extraction was not sufficiently efficient to complete the decarboxylation reaction. Extraction of the cannabis obtained from ONCB Thailand by a group of evaporable solvents tends to yield higher CBD content than THC. On contrary, the supercritical CO_2 solvent yielded a higher content of THC than CBD and the highest content of both THC and CBD among other solvents tested. The vegetable oils provided the lowest amount of both CBD (0.01-0.02%w/w) and THC (0.02-0.03%w/w). For antioxidation assay, CBG, CBN, THC, CBD, and other unidentified compounds showed the activity. Within a group of organic solvents and a group of vegetable oils, the winterized supercritical CO_2 (W-$ScCO_2$) extract and the RBO extract showed the strongest activity with an IC_{50} value of 0.03 ± 0.006 mg/mL and 3.35 ± 0.120 mg/mL, respectively. Furthermore, the ethanol extract, the W-$ScCO_2$ extract, and the VCO extract were tested for their UVA photoprotective effect on HaCat cells. It was found the W-$ScCO_2$ extract significantly protected the UVA-irradiated cell whilst the VCO extract as well as pure CBD and THC showed an insignificant effect.

Field of Study:	Pharmaceutical Sciences and Technology	Student's Signature .....
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Nur Alfan Muhammad Zen

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LIST OF ABBREVIATIONS

µL	Microliter
λ	Lambda
%	Percentage
BP	Boiling Points
CBD	Cannabidiol
CBG	Cannabigerol
CBGA	Cannabigerolic Acid
CBN	Cannabinol
cP	Centipoise
CRCE	CBD-Rich Concentration Extract
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOH	Ethanol
FBS	Fetal Bovine Serum
Hex	Hexane
HH-Olive	High-Heat Grade Olive Oil
HP	Heavy Petroleum
HPLC	High-performance Liquid Chromatography

HPTLC	High-performance Thin Layer Chromatography
LP	Light Petroleum
ng	Nanogram
nm	Nanometer
ONCB	Office The Narcotics Control Board
PSO	Perilla Seed Oil
RBO	Rice Bran Oil
RF-Olive	Roasting & Frying Oilive Oil
$SCCO_2$	Supercritical CO_2
SIO	Sacha Inci Oil
SSO	Sesame Seed Oil
THC	Tetrahydrocannabinol
THCA	Tetrahydrocannabinol Acid
TLC	Thin Layer Chromatography
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
VCO	Virgin Coconut Oil
W-$SCCO_2$	Winterized Supercritical CO_2

CHAPTER I

INTRODUCTION

Due to the legality, ethical, and social implication of its use, *Cannabis sativa* L. is one of the most contentious plants to date. The harmful health effects can even lead to death from consuming this plant for benefit of therapeutic indications (Bridgeman & Abazia, 2017). It is reported that more than 400 known constituents from 18 different chemical groups are contained in this plant (Brenneisen, 2007). The pharmacological effects are accounted for by the bioactive constituents present in this plant. Numerous studies have reported that cannabis has an antimicrobial activity on negative-positive gram bacteria (Monika et al., 2014), anticancer activity (Carter et al., 2011; Fowler et al., 2010; Pertwee, 2012; Ribeiro Grijó et al., 2019; Velasco et al., 2012), and analgesics effect (Romero-Sandoval et al., 2017). As the public enthusiasm for this plant increased, research, especially for medical purposes, expands over the years and it is crucial to accentuate research on the phytochemical composition of *C. sativa* and its products to reveal the expanded potential use of medical cannabis.

One of the potential benefits of cannabis is related to human skincare as seen in many commercial skincare products. The effect on the skin is then speculated. Skin UV protection is of interest being investigated for many skincare products. Sunlight radiation's effects on human health have been a polemical topic in the medical research community. Based on a dermatologist's perspective, sunlight is an ultraviolet radiation source, which can cause skin health problems such as harmful photoaging, immunosuppression, and skin cancer if exposed to excess (Heiskanen et al., 2020). Ultraviolet (UV) light is grouped as UV-$A_{320\text{-}400\ nm}$, UV-$B_{290\text{-}320\ nm}$, and UV-$C_{100\text{-}290\ nm}$. The UV-A light is a primary factor in skin aging and damage, and

it perforates deep into the dermis, which can produce Reactive Oxygen Species (ROS) and latterly generates oxidative stress (D'Orazio et al., 2013; Kuo et al., 2020). ROS or well-known as free radicals and can also be defined as an unstable species that have unpaired electrons chemically. When an imbalance occurs due to the formation of excessive ROS which can't be neutralized through the innate antioxidant defense mechanisms, oxidative stress develops (Poljšak & Dahmane, 2012). Recently, antioxidants preparation has become a trend in increasing the antioxidant defense system to get over the oxidative damage induced by ultraviolet radiation from sunlight (Hassan et al., 2013).

Plant antioxidants are a natural reservoir of the bioactive compound that plays an essential role in plant acclimatization and adaption to environmental aggression but are also helpful for human health (Llauradó Maury et al., 2020). Cannabis could be a candidate to play this function since its antioxidant activity was reported. One of them is the study conducted by Muscarà et al (2020) in evaluating the antioxidant properties and free radical scavenging activity of both standardized hexane extracts obtained from the new Chinese accession of *C. sativa* L. The results indicate that both have strong antioxidant properties and have the ability in DPPH free radical scavenging. Concerning the extraction method directly affecting the group of natural products being extracted, there are ways to extract cannabis. The most common one is using organic solvents. Some organic solvents frequently used to perform in the cannabis extraction process by using maceration technique and solvent evaporation are methanol, hexane, ethyl acetate, toluene, trimethylpentane, ethanol, chloroform, and dichloromethane (Aazza, 2021; Laznik et al., 2020; Webster Barrie & Sarna, 2002). Nowadays, solvents in the chemical industry are used in massive quantities. Thus, solvents exemplify the main part of the environmental performance of a process and impact safety, cost, and health issues.

The innovation of green solvents deliberates the purpose of decreasing the environmental appulse because of solvent utilization in chemical production (Capello et al., 2007). The use of supercritical fluid extraction (SFE) and Vegetable oil as a solvent or co-solvents which are the newest innovative techniques applied in extracting the various bioactive compound from natural products has been widely reported (Deka & Swami Hulle, 2021; Japón-Luján et al., 2008; Ludwig et al., 2021; Paduano et al., 2014; Rosas-Quina & Mejía-Nova, 2021; Sun & Temelli, 2006; Tiono et al., 2021; Yara-Varón et al., 2017). Among the green solvents used, today SFE seems to be the most common extraction method for cannabis (Aiello et al., 2020; Devi & Khanam, 2019; Grijó et al., 2018; Kornpointner et al., 2021; Rochfort et al., 2020).

Infusion or maceration of the aromatic plant even medicinal plant which used vegetable oil as a solvent dates back to ancient civilizations has been proved empirically. Over the years, vegetable oils which been fit for human consumption were efficaciously enriched or aromatized with bioactive compounds from herbs, spices, or various plant substances, therefore, they can improve their nutritional values, organoleptic characteristic, and extend their shelf-existence as properly (Yara-Varón et al., 2017). Virgin coconut oil, olive oil, perilla seed oil, sacha inchi oil, sesame seed oil, and rice bran oil are well-known as vegetable oil which are generally used for households due to their components that supported their uses for cooking oil as well as other benefits for human health such as antioxidant. Several studies have been evaluated the physicochemical properties of each vegetable oil (see Appendix page 100).

Interestingly, there is an approved drug product from cannabis in Thailand namely Deja Formula (Ganja oil) that used Thai local wisdom by heating and frying the cannabis flower in coconut oil. To date, the Deja formula is officially manufactured by the Department of Thai Traditional and Alternative Medicine, Ministry of Public Health, Thailand for several diseases treatment in the hospital.

Notwithstanding the various innovative extraction techniques for *C. sativa* L. that have been developed and implemented by previous reports, studies on using various vegetable oils as a solvent in cannabinoids extraction and its antioxidant activity are still lacking. Therefore, the present study focused on evaluating the quality of cannabis extracts concerning the influence of various vegetable oils as green solvents compared with standard solvents such as supercritical CO_2 and different organic solvents simultaneously and its UVA-Photoprotective effect using human keratinocytes (HaCat) cell lines. In this study, the following objectives have been put forwards:

1. To evaluate the influence of vegetable oil in cannabinoids extraction
2. To investigate the potential of cannabis vegetable oil extract as a photoprotector against UVA radiation on human skin keratinocytes

CHAPTER II

LITERATURE REVIEW

2.1 Morphological Characteristic of *C. sativa* L.

Marijuana, or well-known scientifically as *C. sativa* L. (cannabaceae) being one of the primeval annual herbs cultured since ancient times, is now widely distributed throughout the world. This plant has long been used primarily as a source of food and traditional medicines. Several other benefits such as intoxicating resin and textile fibers have also been reported (Bonini et al., 2018; Kriese et al., 2004; Zuardi, 2006). Cannabis is mostly a dioecious plant. The life cycle of Cannabis is arduous and perplexing, especially at the early growth stage (juvenile); on the contrary, misidentification becomes one of the problems arising from the intricacy in morphological identification between male and female plants. Nevertheless, several developing molecular techniques have succeeded in finding specific differences amid male and female plants which can be identified at the early growth stage (Flachowsky et al., 2001; Sakamoto et al., 2005; Sakamoto et al., 1995; Törjék et al., 2002).

C. sativa is the only genus that is grouped into the cannabaceae. The problem regarding the number of species grouped into the cannabis genus is still controversial, which is supported since various studies have proposed it as a polytypic plant. However, some botanists categorized cannabis as a type of polymorphic plant and even tend to be classified as a monotypic plant. The classification of cannabis according to Linnaeus (1753) as a single species whereas Lamarck (1785) also argues that “Indian Cannabis” is taxonomically different from European Cannabis. Lamarck also gives the specific name as *C. indica* (Chandra et al., 2017). *C. sativa* L. usually grows to 50-100 cm covered with appressed hairs (stem) (Bussmann et al., 2019). In general, it is structured of 5-7 leaflets of cannabis leaves

(**Figure 1**) which are linear-lanceolate, serrated edges, and tapering at two ends. The male flowers (**Figure 2a**) are characterized by the absence of petals. It has five anthers, five yellowish tepals, and axillary or terminal panicles. Sprouts in the axils and terminated with one single-ovulate closely adherent perianth are characteristic of female flowers of this plant (**Figure 2b**). This is emphasized by a single, small, smooth, light brownish-grey fruit that is abundantly composed on each flower.

Moreover, *C. sativa* also has trichomes abundantly located in the leaves and stems of the plant covered by epidermal gland protrusions (Happyana et al., 2013; Huchelmann et al., 2017). Secondary metabolites produced by glandular trichomes known as phytocannabinoids are used for protecting themselves from herbivores and interact with other organisms and are characterized by their distinctive aroma in the presence of terpenoid (Andre et al., 2016). Varieties and climatic conditions have an impact on plant morphology. This plant grows as a persistent weed in soils with a high amount of nitrogen. Cannabis germinates and the feathers split openly, reveal roots, and two coiled embryonic cotyledons leave after 8-12 days during the growth phase of this plant. Moderate levels of environmental and soil moisture and supported by good light intensity are prerequisites for cannabis growing conditions. In 6-12 weeks, cannabis grows vertically and produces new leaves, mostly in the pre-flowering stage with new branches and knots. At this stage, cannabis requires slightly dim lights (Raman & London, 1998).

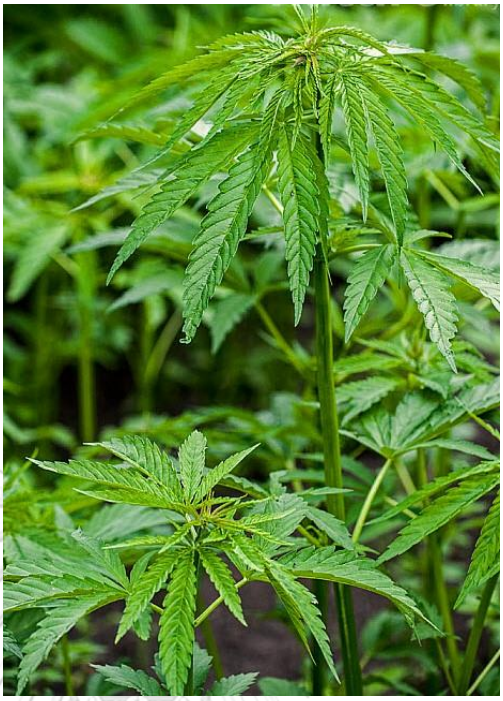


Figure 1. *C. sativa* L. (Philippe Clement in Nature Pic Library)

Figure 2 The flowers of cannabis : (a) Male flower (Dipak Hemraj in leafwell.co); (b) Female flower (Sornkanok Vimolmangkang Picture)

2.2 Phytochemical Constituents of *C. sativa* L.

Secondary metabolite constituents abundantly produced by this plant, cannabis is a complex species chemically based on reported studies. The concentration of Δ^9-THC in the dried inflorescence (leaves and buds) determines the psychoactivity of this plant. The phenotypic and phytocannabinoid profiles of cannabis can be characterized using quantitative and qualitative analysis (Mandolino et al., 1999). Over the last few decades, natural compounds isolated and identified from cannabis have increased. (Turner et al., 1980) has been reported 423 compounds from cannabis and this number upgraded in 1995 to 483 (Ross et al., 2005). The new seven compounds were updated by ElSohly and Slade (2005) between 1995 until 2005 (ElSohly & Slade, 2005). Phytochemical constituents data on 49 cannabinoids currently reported by (ElSohly et al., 2017) are provided in this section. As well as 26 compounds included in the non-cannabinoids group (**Table** 1), 565 compounds have been identified.

Table 1. List of cannabinoids and non-cannabinoids (ElSohly et al., 2017)

Chemical Class	2005	2015
Δ^9 -THC type	9	23
Δ^8 -THC type	2	5
CBG type	8	16
CBC type	6	9
CBD type	7	7
CBND type	2	2
CBE type	5	5
CBL type	3	3
CBN type	7	11
CBT type	9	9
Miscellaneous types	14	30
Total cannabinoids	72	120
Total non-cannabinoids	419	445
Total	491	565

2.2.1 Phytocannabinoids

Cannabinoids were isolated and diagnosticated from cannabis exhibited a specific C21 terpene phenolic skeleton. Transformation and derivatives products of this group were also aforethought as cannabinoids. A total of 120 cannabinoids have been isolated since the chemical compound of cannabis was first explored (**Table** 1), which can be grouped into 11 general types : (-)-Δ^9 -trans-tetrahydrocannabinol (Δ^9 -THC) (**Figure** 3a), which was first reported by Gaoni and Mechoulam (1964), (-)-Δ^8 -trans-tetrahydrocannabinol (Δ^8 -THC) (**Figure** 3b) (Hanus & Krejci, 1975), cannabigerol (CBG) (**Figure** 3c) (Gaoni & Mechoulam, 1964; Hanus & Krejci, 1975). Cannabichromene

(CBC) (**Figure** 3d) has been isolated by the research group (Claussen et al., 1966; Gaoni & Mechoulam, 1964), cannabidiol (CBD) (**Figure** 3e) was first isolated by (Adams & Hunt, 1940), cannabinodiol (CBND) is one of the compounds from Cannabinodiol-type **(Figure** 3f) that have been characterized in *C. sativa* It has been reported from (ElSohly & Slade, 2005; Turner et al., 1980), cannabielsoin (CBE) has been isolated (**Figure** 3g) by (Shani & Mechoulam, 1974), cannabicyclol (CBL) was reported (**Figure** 3h) by (Korte & Sieper, 1964), cannabinol (CBN) was reported for the first time (**Figure** 3i) by (ElSohly & Slade, 2005). Originally, cannabitriol (CBT) has been reported in 1966 (**Figure** 3j), but its chemical structure of this compound was elucidated by (Chan et al., 1975), and miscellaneous types (**Figure** 3k).

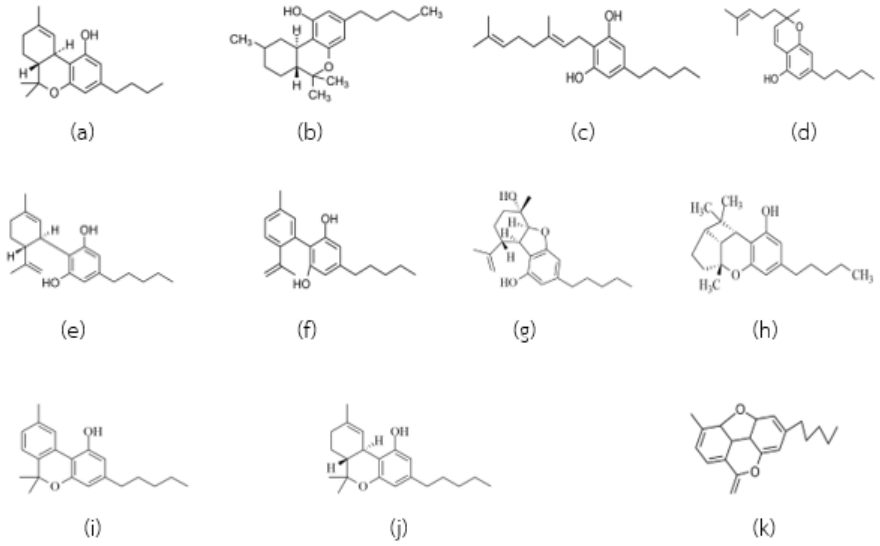


Figure 3 Chemical structure of some cannabinoids : (a) Δ^9 -THC; (b) Δ^8 -THC; (c) CBG; (d) CBC; (e) CBD; (f) CBND; (g) CBE; (h) CBL; (i) CBN; (j) CBT; (k) CBX.

2.2.2 Non-phytocannabinoids

Additionally, various constituents were grouped into various chemical classes that have precedently been explored from cannabis. A total of 419 non-phytocannabinoid constituents, 26 non-cannabinoids were isolated separately from

419 that had been known since 2005. Cannabisin A (**Figure** 4a) is one of the non-cannabinoids constituents that has been isolated from the fruits of cannabis by (Qian et al., 2009). (Yan et al., 2015) reported Cannabisin M (**Figure** 4b) and Cannnabisin N (**Figure** 4c) were isolated from the seed of cannabis.

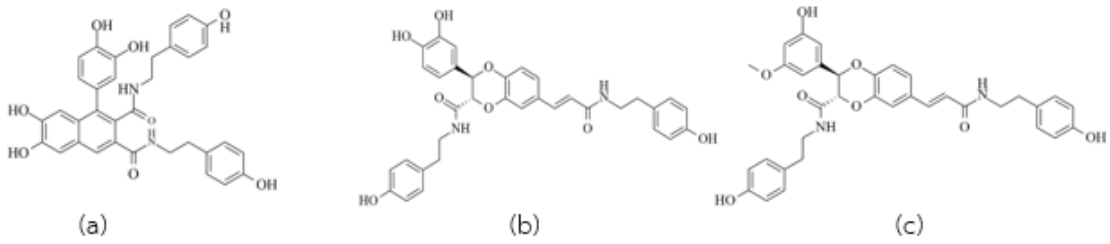


Figure 4 Chemical structure of some non-cannabinoids: (a) Cannabisin A; (b) Cannabisin M; (c) Cannabisin N.

2.3 Biological Activities & Clinical Studies of *C. sativa* L.

For centuries, cannabis and its derivatives have been used for recreational and medical purposes with millions of common users worldwide. Ethnopharmacologically, the use of cannabis as traditional medicine has existed since 5000 years ago recorded in Chinese at the time of Cheng Nan Emperor (Friedman & Sirven, 2017; Russo, 2017). Cannabis as an antibacterial has been reported where ether and acetone extract from cannabis leaf have antibacterial activity against dental microflora (Karas et al., 2020; Raina et al., 2020). Latterly, (Kosgodage et al., 2019) demonstrated CBD as a novel modulator of bacterial membrane vesicles. This study showed a synergistic interaction against *S. aureus*.

(Rodriguez-Martin et al., 2020) have proven in research that protein hydrolysates of *C. sativa* can promote an anti-inflammatory response in primary human monocytes. The study demonstrated that the pro-inflammatory mediators (TNF-α, IL-1β, and IL-6) and anti-inflammatory mediators (IL-10 and IL-4) are

controlled by HPP by reducting pro-inflammatory mediators (TNF-α, IL-1β, and IL-6) and escalating anti-inflammatory mediators (IL-10 and IL-4). HPPs were also degrading M1 polarization marker gene expression (CCR7 and iNOS) and upregulating the M2 polarization marker gene expression (CD200R and MRC1). Eventually, the mRNA expression of chemotaxis genes (CCR2 and CCL2) was downturned by HPPs.

Hydroxycinnamic acid derivatives and cannabinoids isolated from seed, flour, and oil of cannabis (fedora hemp cultivar) have antioxidant activity and investigated the total phenolic content. This study was conducted by (Moccia et al., 2020) which found that seed and flour extract from cannabis did not interrupt the growth of Caco-2 and HT-29 cells. Whereas, cell viability decreased significantly after 24 h of treatment on the cell with oil extract-treated. Activation of apoptotic cell death was associated. However, it is not related to the antioxidant capacity contained in oil extract. This plant has also reported protecting LDL from Cu^{2+}-mediated oxidation. The cannabis sample which used in this study is fresh samples of female inflorescences from three stable *C. sativa* phenotypes (Musetti et al., 2020). The result of other studies also indicated that the essential oil obtained from two non-psychotropic of cannabis had anti-acetylcholinesterase and neuroactive effects. This is attributed to the antioxidant properties of the hydroxylated compounds and significant anti-acetylcholinesterase activities of both samples. Furthermore, it can be concluded that the fibrante type showed the best activity which was indicated by the inhibitory activity which showed a concentration-dependent inhibition of the human and mouse neuronal network's spontaneous electrical activity (Smeriglio et al., 2020). (Rožanc et al., 2021) also reported that cannabis extract can decrease the cell viability of colon cancer cells. CBD and THC which are known as major cannabinoids in *C. sativa* also reported with dose-dependent manner can inhibit the proliferation and viability cell of glioblastoma multiform cancer cells with IC_{50} value range 1 to 8.5 μM and also can inhibit motility and colony formation of this cancer cell (Deng et al., 2017; Peeri et al., 2021). (Nguyen et al., 2021) reported in their study

that cannabidiol from *C. sativa* can block SARS-CoV-2 replication in lung epithelial cells potently.

A growing number of clinical studies indicated that cannabis or a single cannabinoid might have medicinal value for certain diseases and under certain conditions is growing. Clinical studies of cannabis on chronic pain, multiple sclerosis, irritable bowel syndrome, Crohn's disease, pulmonary disease, and Parkinson's disease have been conducted. (Chagas et al., 2014) completed their clinical study regarding the ability of CBD to treat Parkinson's disease in 21 patients with idiopathic PD. Statistically significant differences in UPDRS scores, plasma BDNF levels, or H1-MRS measurements were not found. However, the mean scores obtained for the placebo-treated and CBD groups of 300 mg/day in the placebo-treated groups differed significantly from those of PDQ-39 ($p=0.05$). Briefly, improvements in well-being are significant with no effects on motor functioning or neuroprotection. Naftali and her research team (2013) have also tested the effects of cannabis administration in 21 patients with Crohn's disease. Albeit the final point of their study (induction of remission) was not achieved. However, administration of cannabis with THC-rich within 8 weeks provided significant clinical, steroid-free benefits for 10 of 11 patients with active Crohn's disease placebo as a comparison, without side effects (Naftali et al., 2013). A pilot study of the efficacy and safety of cannabinoids in cases of the pulmonary disease have conducted by (Pickering et al., 2011). The result showed that there was no significant decrease in breathlessness but symptoms such as unpleasantness decreased.

Various studies have been conducted on the evidence of cannabinoids in reducing chronic pain, one of which is (Ware et al., 2010). The study showed a significant reduction in pain, improved sleep quality, and also a reduction in anxiety in 21 patients with neuropathic pain. (Wong et al., 2012) also tested the effects of cannabinoids on 75 patients with IBS. The study was conducted by giving one oral THC at a dose of 2.5 mg and 5 mg. The results obtained show that there is a

reduction in fasting colonic motility in the subgroup of patients. (Zajicek et al., 2012) in their study completed oral administration of cannabis extract in 279 patients with stable muscle stiffness in 12 weeks of treatment with a maximum daily dose of 25 mg THC. The results showed a significant reduction in muscle stiffness. Significant results were also reported in a study of anxiety in 24 patients with SAD by (Bergamaschi et al., 2011) . A single administration of 600 mg of CBD orally with anxiety associated with public speaking in treatment as an indication succeeded in reducing the level of anxiety. Promising results were also confirmed by (Crippa et al., 2011) in 10 patients with SAD. CBD administration at a dose of 600 mg with social indications of rCBF anxiety can reduce the anxiety associated with altered activity in limbic and paralimbic brain areas.

Other clinical studies in the use of CBD-based products (Epidiolex®) have been conducted by (Szaflarski et al., 2018) on 132 patients (60 adults; 72 children) with Epilepsy. The result exhibited significant improvements in Chalfont Seizure Severity Scale (CSSS), (Adverse Events Profile (AEP), and Seizure Frequency (SF) after Epidiolex® administration (5-50 mg/kg/day for 12 weeks which maintained until 48 weeks. The efficacy of CBD for epilepsy treatment was also proved by Kaplan et al (2017). Their study found about 50% reduction in seizures, improve quality of life after administration of CBD (5-25 mg/kg/day) for 14 weeks which was maintained until 63-80 weeks (Kaplan et al., 2017). Positive results have also been successfully applied to patients with childhood-onset refractory epilepsy. Of 66 patients, 48.5% improvement in seizure burden (around more than 50%), 21.2% (seizure-free) with none to mild side-effect (Neubauer et al., 2018). (Good et al., 2019) have reported the reduction of symptoms in patients with advanced cancer after oral administration of CBD (100 mg/mL; dose 50-600 mg/day).

2.4 Extraction Method of *C. sativa* L.

The use of cannabis by humans as herbal medicines dates back to ancient times. Cannabinoids are the most sought-after target compounds because its pharmacological effects which can be used to cure various diseases. More than 100 cannabinoids have been isolated to date resulting in high chemical complexity. Generally, cannabinoids are produced in almost all of the main organs of cannabis such as flowers, leaves, and stems which grow in an acidic form known as THCA, CBGA, and CBDA. However, there are not many studies that intensively prove the pharmacological effects of cannabinoids acid lately. Cannabinoids acid is considered an inactive compound that will be activated by the decarboxylation process (Hartsel et al., 2016; Moreno et al., 2020; Wang et al., 2016). Decarboxylation is a natural process that occurs continuously. In general, this process is slow but exposure to heat, light, and oxygen can speed up the process (Veress et al., 1990). Several studies regarding the cannabis extraction method have been reported.

Recently, ethanol is one of the organic solvents most often used in the extraction method. Hong et al (2015) reported in their study the use of ethanol as a solvent in the cannabis extraction process (Hong et al., 2015). The use of ethanol was also reported by Yoon et al (2018) in cannabis extraction (Yoon et al., 2018). The supercritical fluid extraction method has also become a popular method since it was developed decades ago in plant extraction, cannabis specifically. This method was developed with the principle of heating above the critical temperature and pressure of the solvent used. The specificity of the technique depends largely on the physicochemical properties of the main solvent used (Attard & Hunt, 2018; Baldino et al., 2020; Chemat et al., 2020). CO_2 is the most solvent used in this method. However, the use of CO_2 is considered to produce greenhouse gases as a byproduct when used on a large scale even though it is non-toxic and non-flammable (Radoiu et al., 2020). Aiello et al (2020) are among those who have used the supercritical CO_2 method in extracting cannabis (Aiello et al., 2020).

The extraction of cannabis using liquefied gases such as n-propane and n-butane which are both good solvents for low polarity cannabinoids is a fairly popular method in cannabinoid extraction. This method is well-known as the hydrocarbon extraction method. The gases used in this method will remain in the liquid phase at low pressure (2-10 bar) therefore can minimize solvent residues. Gallo-Mollina et al (2019) extracted THC from cannabis using this method (Gallo-Molina et al., 2019). Unlike, the Microwave-assisted Extraction (MAE) method, this method uses volumetric heating as opposed to transferring heat from the surface to the inside. Therefore, the extraction process is more efficient because the temperature and contact time can be completely controlled (Veggi et al., 2013). Fiorini et al (2020) reported the use of the Microwave-assisted Extraction method in extracting CBD-enriched cannabis essential oil (Fiorini et al., 2020). Apart from cannabinoids, the phenolic compound has been extracted from cannabis by using this method (Matešić et al., 2020).

2.5 Vegetable Oils

2.5.1 The major components of vegetable oil

In general, vegetable oil is obtained from plant seeds or fruits simply by pressing and/or extracting by using solvents. Triglycerides (**Figure** 5) are not the only component in Vegetable oils. Chemically, Vegetable oil is structured with three fatty acid molecules esterified to one glycerol molecule which makes up about 95-98% of Vegetable oil. The classification, positions, and proportions of fatty acids on the glycerol backbone specify the characteristic of triglycerides. Varieties, cultivation, agronomic, and climate condition are the main factors affecting the fatty acid composition of triglycerides in Vegetable oil. Vegetable oil's bio-syntheses pathways cause the even-numbered carbon atoms to monopolize the fatty acid chains' length. The variation in chemical and physical properties is the effect of the saturation degree of fatty acids in Vegetable oil which may have different configurations.

Generally, the saturation degree can be grouped into saturated, mono-unsaturated, and poly-unsaturated fatty acids, which may have different configurations, resulting in various physical and chemical properties (Yara-Varón et al., 2017).

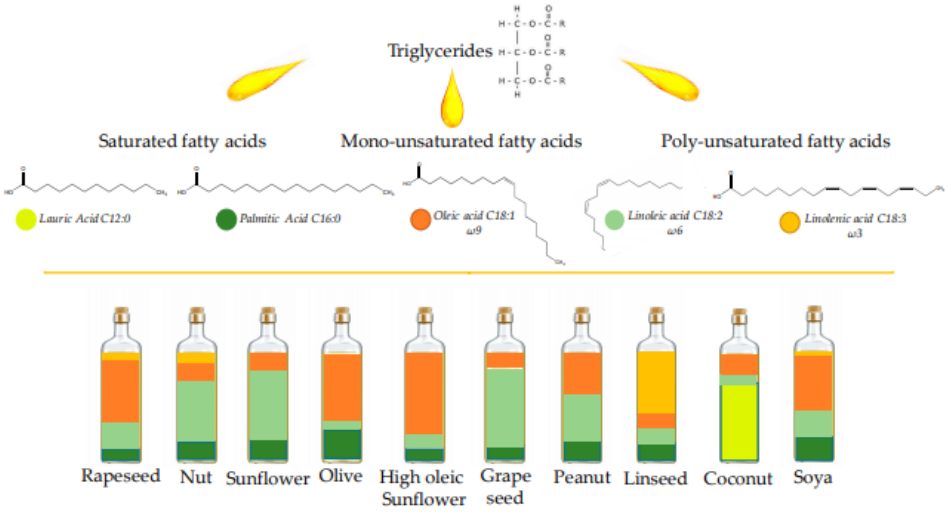


Figure 5 The major components of vegetable oil

2.5.2 The minor components of vegetable oil

Due to its biological properties and nutritional value, which are very attractive to the pharmaceutical and nutraceutical industries, the composition presentation of minor components, which is only less than 5%, cannot be ignored, particularly in virgin oil (Purcaro et al., 2016). The minor components can be parted into two groups: glycerolipids such as monoglycerides and diglycerides, phospholipids, and non-glycerolipids such as sterols, tocopherols/tocotrienols, pigments, free fatty acids, vitamins, phenolic compounds, proteins, water, etc. (**Figure** 6) (Aluyor et al., 2009; Chen et al., 2011). Commonly during the refining process, some unwanted components are customarily removed by food manufactures. However, the refining process will not run efficiently and selectively which results in colloids formed from remaining unwanted minor components in Vegetable oil (Kittipongpittaya et al., 2014).

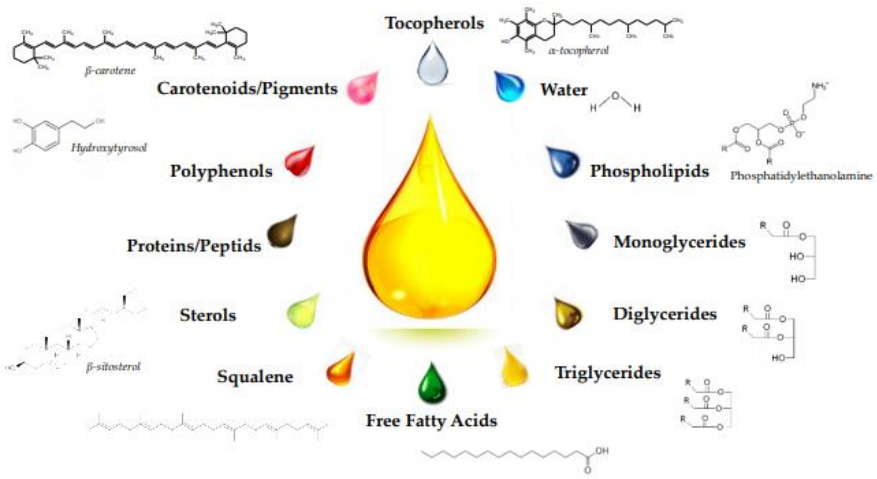


Figure 6 The minor components of vegetable oil (Yara-Varón et al., 2017)

2.5.3 Virgin Coconut Oil (VCO)

Virgin Coconut Oil (VCO) is an edible oil obtained from the extraction of fresh coconut kernel without chemical treatment such as refining, bleaching, or deodorizing which in other words is wet processing via coconut milk (Rohman et al., 2021). Physically, VCO almost looks colorless (clear) with a sour smell slightly and has sweet and salty tastes (Villarino et al., 2007). Chemically, lauric acid (C12:0) (**Figure** 7) is a fatty acid that dominates in Virgin Coconut Oil composition (Gaston et al., 2021). Refers to literature, VCO showed some benefits for human health due to its pharmacological effect such as antibacterial, antiviral, antifungal, antiparasitic, cardioprotective, hepatoprotective, antidiabetic, hypolipidemic, anti-inflammatory, and also antioxidant (Akula et al., 2021; Illam et al., 2021; Iranloye et al., 2013; Janu et al., 2014; Lokesha, 2021; Lopez et al., 2021; Salian & Shetty, 2018).

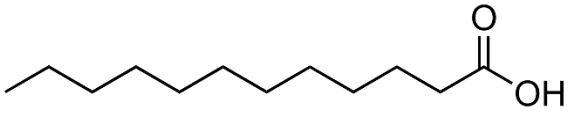


Figure 7. Lauric acid (C:12)

2.5.4 Olive Oil (OO)

Olive oil is an oil derived from the extraction of olive (*Olea europaea*) which has long been known in the Mediterranean diet as a source of lipids. Olive oil is also grouped into some types due to the extraction method and the quality for commercial consumption including extra virgin olive oil (EVOO), virgin olive oil (VOO), olive oil (OO), and olive pomace oil (OPO). The chemical component of olive oil is dominated by MUFA which is Oleic acid (C18:1) (**Figure** 8) 60-80% which depends on a variety of *Olea europaea* (Jimenez-lopez et al., 2020). Several studies confirmed that olive oil consumption results in benefits whether for human health or disease prevention such as antioxidants (Bartolomei et al., 2021; Kouka et al., 2017), anticancer (Buckland et al., 2012; Owen et al., 2004; Psaltopoulou et al., 2011), antibacterial (Karygianni et al., 2019; Nazzaro et al., 2019), and antidiabetic (Jurado-Ruiz et al., 2019; Yubero-Serrano et al., 2019). The sensory profile of olive oil is also affected by the variety of olive, climatic, and extraction methods where the sensory profile has a direct impact on economic value (Silva & Schmiele, 2021).

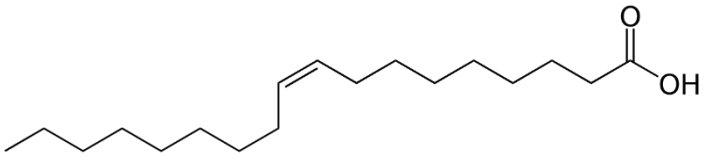


Figure 8. Oleic acid (C18:1)

2.5.5 Sacha Inchi Oil (SIO)

Sacha Inchi Oil (SIO) is an oilseed that was extracted from the seed of sacha inchi or well-known scientifically as *Plukenetia volubilis* that cropped in the Amazon basin (Kodahl & Sørensen, 2021). Like other vegetable oil, the fatty acid still dominates the chemical composition which **α**-linolenic acid (C18:3) (**Figure** 9) dominates up to 50% of the fatty acid of sacha inchi oil (Keawkim et al., 2021). Suwanangul et al., 2021 reported that protein hydrolysates and its fractioned peptides can inhibit several digestive enzymes such as **α**-amylase, **α**-glucosidase, pancreatic lipase activity (Suwanangul et al., 2021). The antibacterial properties of SIO were also proven by (Gonzalez-Aspajo et al., 2015). A study from Rincón-Cervera et al (2016) exhibits that **α**-linolenic acid which found a high amount in SIO has contributed to the antioxidant activity of SIO (Rincón-Cervera et al., 2016). Lipid profile in hypercholesterolemic patients after SIO intake was evaluated which the result showed a decrease in Total Cholesterol (TC), Non-Esterified Fatty Acids (NEFA), LDL, and enhancement of insulin level and HDL (Garmendia et al., 2014; Gonzales & Gonzales, 2014).

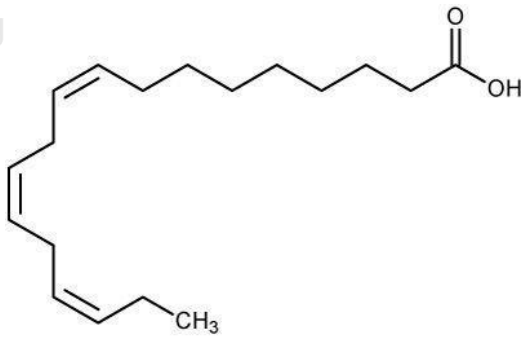


Figure 9. **α**-linolenic acid (C18:3)

2.5.6 Perilla Seed Oil (PSO)

Perilla seed oil (PSO) is an oilseed obtained from *Perilla frutescens* seed which contains five major fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, and **α**-linolenic acid. **α**-linolenic acid (**Figure** 9) is a fatty acid that dominates 54.3-70.4% of the overall fatty acid composition of PSO (Park et al., 2021). The odor of PIO is an aromatic special, therefore that is not suitable for the preference of some consumers (H. Wang et al., 2021). Apart from being a good source of PUFA, PSO also has various benefits for human health which are proven by several studies due to its pharmacological activities such as anti-tussive (Zhang et al., 2021), anti-inflammatory (Kangwan et al., 2021), antioxidant (Hashimoto et al., 2021), antidepressant-like effect (Ji et al., 2014), and antibacterial (Li et al., 2018; H. Wang et al., 2021).

2.5.7 Sesame Seed Oil (SSO)

Sesame (*Sesamum indicum*) is known as a queen of oilseeds and as one of the oldest crops which is partly used for oil production (Islam et al., 2016; Moazzami & Kamal-Eldin, 2009). Linoleic acid (C18:2) (**Figure** 10) and oleic acid (C18:1) (**Figure** 8) are the major fatty acids composed in sesame seed oil which cover up to 60% with almost equal levels (Wacal et al., 2019). Several studies have been showing the pharmacological effect of sesame oil. (Alshahrani et al., 2020) exhibit antibacterial properties against some pathogenic bacterias. Other benefits of sesame oil also proved such as anti-inflammatory (Deme et al., 2018), anticancer (Majdalawieh & Mansour, 2019), and antioxidant (Mekky et al., 2019).

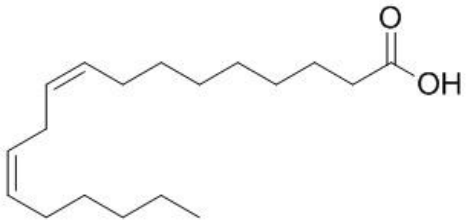


Figure 10. Linoleic acid (C18:2)

2.5.8 Rice Bran Oil (RBO)

Rice (*Oryza sativa*) processing with various processes will produce by-products in various forms of broken rice, germs, husks, and the most interesting is the bran which although usually discarded or used as animal feed and only 9% of the total weight of rice but the nutritional contribution of whole rice grain can reach 65%. To date, the most commonly used rice bran is an oil source that contains 18-22% of oil and various bioactive constituents as well (Ardiansyah et al., 2006; Khatoon & Gopalakrishna, 2004; Rohman, 2014; Saikia & Deka, 2011). The fatty acids component in RBO is composed of unsaturated fatty acids where MUFA represented oleic acid (C18:1) (**Figure** 8) and PUFA represented by linoleic acid (C18:2) (**Figure** 10) almost has a percentage in fatty acid composition of RBO equally 35-40% (Lai et al., 2019). Various chemical components contained in RBO greatly affect the pharmacological effects caused. The hepatoprotective effect of RBO has been reported by (Phannasorn et al., 2021). Improvement on hyperglycemia control and lipid profile in patients with diabetes mellitus type 2 after RBO blended with sesame oil (80:20) intake was also reported (Devarajan et al., 2016).

2.5.9 Vegetable Oils as an Alternative Solvent

Nowadays, there have been many reports regarding the use of vegetable oils as an alternative solvent. In 2001, Damechki et al have successfully tried using

rosemary and oregano gourmet oil in their research. The result showed that there has been a 1.7 and 3.5 times increase in total phenol content in the rosemary and oregano gourmet oils sample (5 wt %, 24, 48, 72 h agitation, dark) which have superior oxidative stability and consumer acceptability to the origin oil (Damechki et al., 2001). Garcia-Martinez et al (2014) also succeeded in evaluating the effect of olive variety and elevation of orchards on the content of phenolic compound Sicilian virgin olive oils (VOOs). The effect of the phenolic extract on osteoblast cell growth using the human MG-63 osteosarcoma cell line was also being part of this study. The result showed that there were variations in the phenolic content produced by olive oil according to the type of cultivar and grove altitude as well as an effect on human osteosarcoma cell proliferation (García-Martínez et al., 2014). (Romeu et al., 2016) also evaluated the protective effect against oxidative DNA damage and antioxidant endogenous enzymatic system (AEES) of virgin olive oil (VOO) enriched with phenolic compounds and/or thyme phenolic compounds. This experiment was applied to 33 hyperlipidemic subjects after the consumption of VOO, VOO enriched with PC (FVOO), or VOO complemented with thyme PC (FVOOT). The protective effect test in this study showed that FVOOT gave a significant effect in protecting from oxidative DNA damage and also improving the AEES. Continued consumption of FVOOT enhances the protective effect of DNA against oxidation and AEES. Greater bioavailability of thyme phenolic compound in hyperlipidemic subjects possibly increases the protective effect. The use of vegetable oil as a solvent in cannabis extraction has been carried out by (Hazekamp & Romano, 2013) using olive oil by heating the sample during extraction with varying concentrations and temperatures of olive oil. The result of this study proves that the use of olive oil provided good performance in extracting several cannabinoids, especially THC and CBD (Casiraghi et al., 2018).

2.6 Human Skin

Skin is the largest and main protector of all the vital organs inside the human body. The skin covers its entire external surface and serves as a first-order physical barrier against interference from outside. The skin has other functions such as temperature regulation and as a protector from ultraviolet, protection from pathogens, trauma, toxins, and microorganisms. Immunological superintendence, control of unconsciously fluid loss, and homeostasis generally are acted on by the skin. Adaptations to the thickness and special functions of human skin are likely to occur in various sites of the body (Maranduca et al., 2019; Someya & Amagai, 2019). Human skin dynamically regenerates itself by prepossessing outside-in-body in the human body (Baroni et al., 2012). Keratinocytes embody 95% of the epidermal cells. Functionally and structurally, keratinocytes are not only protecting the epidermis but preserving the inflammatory and immunological response of the skin, and wound-healing is also played by keratinocytes (Hänel et al., 2013). Under homeostatic conditions, keratinocytes differentiate and mature from proliferating nucleated basal cells to the profoundly differentiated (corneocytes). Expressed structural proteins such as keratin and lipids indicate each phase of differentiation (Proksch et al., 2008).

2.7 Biological Effect of Ultraviolet (UV) Radiation on Human Skin

Cosmic radiation, gamma rays, x-rays, infrared radiation, and ultraviolet radiation are the amounts of energy emitted by the sun. The atmosphere that surrounds our earth will reflect all the high-energy cosmic, gamma, and X radiation. The stratospheric layer absorbs most of the ultraviolet radiation efficiently. Ultraviolet C (200-280 nm) does not reach the earth because it has been engrossed by the ozone layer but ultraviolet C cause skin-burning and skin cancer in humans as well as ultraviolet B (290-320 nm) is also absorbed by the ozone layer and only spreads the earth's surface about 0.1% in the summer (noon-time). Ultraviolet B is also absorbed by the epidermis on human skin. Excessive ultraviolet B radiation also

causes tanning, sunburn, photoaging, and wrinkles. The carcinogenic properties effect of ultraviolet B radiation is a thousand times more than ultraviolet A radiation. About 90-99% of ultraviolet A radiation (321-400 nm) reaches the earth's surface. The risk of overexposure has been considered harmful. Overexposure can promote pigmentation and skin aging (Holick, 2016; Narayanan et al., 2010). Ultraviolet exposure into the skin triggers photochemical reactions which lead to acute conditions such as photoaging, the main factor of changes in the appearance of the skin, and skin cancers such as cutaneous malignant melanoma, basal cell carcinoma, and squamous cell carcinoma (Biniek et al., 2012).

2.8 UVA-Photoprotection

Overexposure to ultraviolet radiation from the sun significantly increases skin cancer and skin aging risk on our body. For a long time, understanding of the mechanism of ultraviolet A in damaging the skin has been advancing. This perception is proofed by the development of sunscreen formulation with skin protection capabilities that extend to ultraviolet A wavelength. The amalgamation of the previous perspective combined with the consciousness that ultraviolet A promotes free radicals has focused new research on the risk of free radicals to skin health (Chen et al., 2012). At the cellular level, Reactive Oxygen Species induces fatty acid peroxidation to the plasma membrane. The peroxidation radicals and fatty hydroperoxides further amplify the oxidative damage. Therefore, antioxidants are the most crucial key in reducing ultraviolet radiation's damaging effects (Amaro-Ortiz et al., 2014). Neutralization of radicals that come from exogenous and endogenous sources can be carried out by our body because it has a built-in antioxidant defense system. However, in an imbalance condition, this antioxidant can be depleted even though our body has an innate antioxidant defense system to neutralize radicals initiated from both the exogenous and endogenous sources, this antioxidant reservoir can be depleted speedily. Henceforward, topical antioxidant supplementation is

urgently needed at least in the literature, promising to provide extra benefits to the skin, outstandingly under oxidative stress as a result of overexposure ultraviolet A radiation (Chen et al., 2012).

CHAPTER III

RESEARCH METHODOLOGY

3.1 Material

3.1.1 Plant Materials

The plant material used for the experiment on solvent effect on cannabinoids profile and UVA-Photoprotection is *C. sativa* L. (adulterated-cannabis flower) were provided by the Office of the Narcotics Control Boarding (ONCB) Thailand and it was grounded into powder and decarboxylated by heating the material at 110°C for 60 minutes in a hot-air oven. While plant material used for an experiment on the decarboxylation effect on the cannabinoid profile is a female flower of *C. sativa* CBD Charlotte's Angel strain.

3.1.2 Chemicals

Vegetable oils used in this study as a solvent including Virgin Coconut Oil (PLEARN®, Thailand), Sacha Inchi Oil (BioTrade Thai, Thailand), Perilla Seed Oil, Sesame Oil, Olive Oil Roasting & Frying Grade (BERTOLLI®, SPAIN), Olive Oil High-heat Cooking Grade (BERTOLLI®, SPAIN), and Ricebran Oil. Organic solvents used for extraction were ethanol, light petroleum ether (BP 40-60°C), heavy petroleum ether (BP 60-80°C), and hexane from SIGMA-Aldrich®, USA. Other chemicals also used in this study are methanol, chloroform, vanillin, sulfuric acid, DPPH (2,2-diphenyl-1-picrylhydrazyl) from SIGMA-Aldrich®, USA. Cannabidiol-Rich Cannabis Extract (CRCE) was the CBD Distillate – Broad spectrum 0% THC from CBD Capital Ltd., Surrey, UK (Cat No. CBD102). Cannabinoid reference standards were CBDA, CBG, THCA, THC (Cayman Chemical Co, USA), CBN, and CBD (THC Pharm GmbH, Germany). Human skin keratinocytes (HaCat cell lines) for photoprotective assay were obtained from Elabscience (Houston, TX, USA).

3.2 Methods

3.2.1 Plant Extraction

3.2.1.1 *C. sativa* L. strain CBD-Charlotte's Angel Female Flower

Before the extraction process, the dried powder of cannabis flower was divided into two groups: i) decarboxylated group (H) by heating the sample at 110°C for 60 min and ii) non-heated sample group (NH). Both groups will be extracted using two sets of solvents where ethanol and hexane represent organic solvent while vegetable oils are represented by virgin coconut oil (VCO) and olive oil. Five grams of cannabis flower powder were extracted using the Ultrasound-assisted extraction method with 3 repetitions at 50°C where the duration of each repetition was 30 minutes respectively. Thereafter, each organic solvents extract that has been collected from 3 repetitions was combined and then filtered using Whatman No. 1 filter paper and evaporated using a rotary evaporator (BUCHI Labortechnik AG, Flawil, Switzerland) to get a crude extract. While each vegetable oil extract that has been collected from 3 repetitions was combined and filtered firstly using cotton. After that, all prepared vegetable oils extract were stored at room temperature for the experiment.

3.2.1.2 *C. sativa* obtained from ONCB Thailand

Five grams of cannabis powder which has been pre-heated at 110°C for 60 minutes extracted using various organic solvents including ethanol, hexane, light petroleum, heavy petroleum, and various vegetable oils including olive oils, virgin coconut oil (VCO), perilla oil, sacha oil, sesame oil, and rice bran oil with a ratio of cannabis powder to solvent (1:10 w/v). Afterward, all the samples will be soaked in organic solvents and vegetable oils respectively for 24 hours. Thereafter, organic solvents extract that has been collected filtered using Whatman No. 1 filter paper and evaporated using a rotary evaporator (BUCHI Labortechnik AG, Flawil, Switzerland) to get a crude extract. While vegetable oils extract that has been

collected filtered firstly using cotton and stored at room temperature for the next experiment.

In addition, supercritical CO_2 extract and winterized supercritical CO_2 extract which are included in this study were extracted by using the Supercritical Fluid Extraction (SFE) method. Sixty grams of dried cannabis powder were added to the extraction vessel. This extraction process lasts for 4 hours at 55°C and 225 bar. The extract was obtained directly from the vessel extractor is supercritical CO_2 extract (wax extract). A further process was carried out to obtain winterized supercritical CO_2 extract (non-wax extract) by mixing an aliquot supercritical CO_2 extract with ethanol (1:10 w/v) then incubated at -20°C overnight. Thereafter, the extract that has been incubated overnight was filtered and then evaporated until getting a winterized supercritical CO_2 extract. The sample details process is summarized in Table 2.

3.2.2 Sample Preparation

3.2.2.1 HPTLC Chemical Profiling

For crude extract preparation, one milligram of cannabis extracts was dissolved in 1 mL of the solvent mixture, methanol : chloroform (9:1, v/v) to get the concentration of 1 mg/mL of sample for analysis. Afterward, the sample was sonicated for 15 minutes then filtered by using a 0.2-micron PTFE filter. Crude extract of supercritical CO_2 and winterized supercritical CO_2 was melted firstly at 80 and an aliquot of the melted extract was then dissolved in the solvent mixture (methanol 9:1 chloroform) to obtain a solution concentration of 100 mg/mL. The sample solution was sonicated for 30 minutes and then filtered using 0.2-micron PTFE filter paper. The filtered sample was then adjusted for HPTLC analysis which is 1 mg/mL. For oil sample preparation, a 200 µL of oil sample mixed with 800 µL of the solvent mixture (methanol 9:1 chloroform) to get 20 mg/mL sample solution and then the mixture will be sonicated for 15 minutes and followed by centrifugation at 3500 rpm

for 5 minutes. The separated supernatant was collected and then filtered using a 0.2-micron PTFE filter paper. The filtrate obtained from the filtering process is stored at room temperature until it will be used for experiments.

Table 2. Summary of *C. sativa* L. extraction

Sample Set	Sample Code	Solvent used
Soaking cannabis powder in various organic solvents and vegetable oils for 24 hours without decarboxylation process (Non-heated)	NH-EtOH	Ethanol
	NH-Hex	Hexane
	NH-Olive	RF- Olive Oil
	NH-Coco	Virgin Coconut Oil
Heating at 110°C the cannabis powder for 60 minutes before the extraction process then followed by soaking the cannabis powder in various organic solvents and vegetable oils for 24 hours	H110-EtOH	Ethanol
	H110-Hex	Hexane
	H110-LPet	Light Petroleum
	H110-HPet	Heavy Petroleum
	H110-RFOlive	RF- Olive Oil
	H110-HHOlive	HH- Olive Oil
	H110-Coco	Virgin Coconut Oil
	H110-Perilla	Perilla Seed Oil
	H110-Sacha	Sacha Inchi Oil
	H110-Sesame	Sesame Seed Oil
	H110-Ricebran	Rice Bran Oil
	H110-ScCO2	SFE - CO_2
	H110-ScCO2 (W)	SFE - CO_2 after Winterization

3.2.2.2 HPLC Analysis

3.2.2.2.1 Cannabinoids Quantification

For crude extract preparation, one milligram of cannabis extracts was dissolved in 1 mL of the solvent mixture, methanol : chloroform (9:1,v/v) to get the concentration of 1 mg/mL of sample for analysis. Afterward, the sample was sonicated for 15 minutes then filtered by using a 0.2-micron PTFE filter. Crude extract of supercritical CO_2 and winterized supercritical CO_2 was melted firstly at 80 and an aliquot of the melted extract was then dissolved in the solvent mixture to obtain a solution concentration of 100 mg/mL. The sample solution was sonicated for 30 minutes and then filtered using 0.2-micron PTFE filter paper. The filtered sample was then adjusted for HPTLC analysis which is 1 mg/mL. For oil sample preparation, a 100 µL of oil sample mixed with 900 µL of the solvent mixture (methanol 9:1 chloroform) to get 20 mg/mL sample solution and then the mixture will be sonicated for 15 minutes and followed by centrifugation at 3500 rpm for 5 minutes. The separated supernatant was collected and then filtered using a 0.2-micron PTFE filter paper. The filtrate obtained from the filtering process is stored at room temperature until it will be used for experiments.

3.2.2.2.2 UVA-Photoprotection Assay

All sample used in this experiment has been re-analyzed for the content of THC that was used as standard using HPLC which has the same step for sample preparation before being used for UVA-Photoprotection assay. All the sample was re-prepared for cell assay dissolved in DMSO. A total of 0.0097 grams of ethanol crude extract was dissolved in 970 µL of DMSO. While 100 µL of supercritical CO_2 and winterized supercritical CO_2 extract was also dissolved in 900 µL of DMSO. Lastly, 100 µL of the VCO sample was dissolved in 900 µL DMSO.

3.2.3 Chemical Profiling

Chemical profiling of all samples using High-Performance Thin Layer Chromatography (HPTLC). All the chemicals used in this experiment are analytical grade. The HPTLC plate used is the HPTLC glass plate $Si60F_{254}$ (10x20) (Merck, Germany) from 0.2 mm thickness is used as the stationary phase. The mobile phases used in the development of plates to separate compounds contained in all extracts are heptane, diethyl ether, and formic acid with a ratio 90:20:0.3 (v/v/v).

Five µL each sample extract and 4 µL of cannabinoids standard applied to a 10 x 20 cm glass plate as a band. The application of the sample on the plate was assisted by the Linomat 5 CAMAG sample applicator with a width of 10 mm each. The plate was developed using the CAMAG Automatic Developing Chamber (ADC). Each plate developed up to 80mm, dry air, and then the plates were placed in the photo room documentation CAMAG Visualizer which captured all the images under white light, UV light at UV_{254}, and UV_{366}. The image scanning and analysis are operated by WINCATS software. Hereinafter, the plates were derivatized using the 1% vanillin reagent followed by heating at a temperature of 105°C on a hot plate until colored bands appear.

3.2.4 HPLC Analysis

3.2.4.1 Cannabidiol Quantification of *C. sativa* CBD-Charlotte Angel's Extract

The quantification of cannabidiol (CBD) was carried out using HPLC with a slightly modified method previously used by Citti et al (2018). All the extracts were analyzed with the Agilent 1260 Infinity II HPLC system (Agilent Technologies, USA), including a flexible pump, vial sampler, thermostat column compartment, and a diode array detector (DAD) WR. EC-C 18 column guard (3.0 × 5 mm, 2.7 µm, InfinityLab Poroshell 110, Agilent) and reverse phase EC-C 18 column (3.0 × 150 mm, 2.7 µm, InfinityLab Poroshell 110, Agilent). The column temperature is 35 °C. This

method uses a gradient elution consisting of 0.1% formic acid in water (A) and acetonitrile (B). Flow rate 0.5 mL/min for 25 minutes. The gradient conditions are carried out as follows; 0-15.0 minutes; 70-80% B, 15.0-15.1 min; 80-95% B, 15.1-18.0 min; 95% B, 18.0-18.1 min; 95-70% B, 18.1- 25.0 min; 70% B. The injection volume used is 5 µL (Citti et al., 2018).

3.2.4.2 Cannabinoids Quantification of *C. sativa* extract obtained from ONCB Thailand

The quantification of Δ^9 – Tetrahydrocannabinol (THC) and cannabidiol (CBD) were carried out using HPLC with a slightly modified method previously used by Citti et al (2018). All the extracts were analyzed with the Agilent 1260 Infinity II HPLC system (Agilent Technologies, USA), including a flexible pump, vial sampler, thermostat column compartment, and a diode array detector (DAD) WR. EC-C 18 column guard (3.0 × 5 mm, 2.7 µm, InfinityLab Poroshell 110, Agilent) and reverse phase EC-C 18 column (3.0 × 150 mm, 2.7 µm, InfinityLab Poroshell 110, Agilent). The column temperature is 35 °C. This method uses a gradient elution consisting of 0.1% formic acid in water (A) and acetonitrile (B). Flow rate 0.5 mL/min for 25 minutes. The gradient conditions are carried out as follows; 0-15.0 minutes; 70-80% B, 15.0-15.1 min; 80-95% B, 15.1-18.0 min; 95% B, 18.0-18.1 min; 95-70% B, 18.1- 25.0 min; 70% B. The injection volume used is 5 µL (Citti et al., 2018).

3.2.5 Antioxidant Evaluation

3.2.5.1 DPPH Colorimetric Assay

The free radical scavenging capacity of each sample was determined by using the slightly modified method of Roheem et al (2020). Concisely, 75 µL of the freshly prepared 0.4 mM DPPH solution was introduced into each 96-wells microplate discreetly.

A 50 µL of sample and standard with various concentrations were added to each well. The blank contains only methanol and DPPH. Standard gallic acid with an initial concentration (1 mg/mL) was dissolved in methanol. The microplates were left in the dark for 30 minutes (Roheem et al., 2020). Hereinafter, the plate will be placed in the microplate reader and the absorbance will be read at $\lambda_{517\ nm}$ using CLARIOStar microplate reader (BMG Labtech, Germany). The assay was performed in triplicate and the % inhibition of each sample/standard was calculated using the following equation:

$$(\%\ \text{DPPH Inhibition}) = \left(\frac{\text{AbsControl} - \text{AbsSample}}{\text{AbsControl}}\right) \text{x}100$$

3.2.5.2 TLC-Bioautography

The TLC plates which have been developed with the same treatment are dried first, then the plates were sprayed with 0.2% DPPH which has been dissolved in methanol using the TLC sprayer. Shortly after spraying with the DPPH reagent, the plates were incubated for 30 minutes in a dark place. Furthermore, the image will be taken using the CAMAG TLC Visualizer 2 (Sobstyl et al., 2020).

3.2.5 UVA-Photoprotection Assay

3.2.5.1 Cell Culture

Human skin keratinocyte cell line, HaCaT cells, was obtained from Elabscience (Houston, TX, USA). HaCat cell cultured by following cell conditions from Warinhomhoun et al (2021) in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA), then 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) were added. The cells

were then stored in an incubator humidified at 37ºC with an atmosphere of 5% CO2 for the next experiment (Warinhomhoun et al., 2021).

3.2.5.2 UVA-Photoprotection Assay

Human Skin Keratinocytes (HaCaT cells) were seeded into 96-well culture plates at a density of 2 x 10^4 cells per well in 200 µL medium and then the 96-well culture plate was incubated for 24 h. Cells were pre-treated with CBD, THC, or extracts for 18 h. Control groups were incubated with an equivalent amount of DMSO (0.5%; non-toxic concentration of DMSO; vehicle control). Before UVA irradiation, experimental media were aspirated; cells were washed twice with PBS, and serum-free medium was added. Hereinafter, cells were irradiated with UVA (365 nm; 7 J/cm^2) using UVP crosslinker CL-3000L (Analytik Jena, Jena, Germany). After irradiation, the survival of cells was evaluated immediately by using an MTT assay then percentage of cell viability calculated according to following formula.

$$\%\ Survival = \frac{OD570Tx\ x\ 100}{OD570Ct}$$

CHAPTER IV

RESULTS & DISCUSSIONS

4.1 Yield Production of *C. sativa* Extract

C. sativa samples used in the first experiment were obtained from the female flower of *C. sativa* L. strain CBD Charlotte's Angel. The two extraction groups were the first group where all the cannabis flower powder had been pre-heated for 60 minutes at 110°C and the second group are cannabis flower powder without pre-heated before extraction then extracted using ethanol and hexane. The extraction method used for this sample is ultrasound-assisted extraction (UAE) which extraction was carried out for three repetitions where each repetition lasts for 30 minutes. The organic solvent extracts obtained from flower extraction have then calculated the percentage of yield which has been presented in Table 3.

Cannabis sample obtained from ONCB Thailand in the second experiment used the cold-maceration method in which the decarboxylated powder was firstly soaked in various organic solvent and vegetable oils overnight then the collected-extract from organic solvents filtered and evaporated to separate the extract from solvent until getting crude extract while collected-extract from vegetable oil filtered and store at room temperature. Additionally, the extract from SFE ($SCCO_2$) has been collected from vessel extraction then winterized to get a winterized-$SCCO_2$ sample. The organic solvent extract and supercritical CO_2 extract obtained from extraction have then calculated the percentage of yield which has been presented in Table 4. Since we did not vaporize the vegetable oil from the extract due to the component of vegetable oil which contains heavy molecules with a long carbon chain that make oil does not evaporate even at high temperatures, therefore we did not provide the yield data of vegetable oil samples.

Table 3. Yield production of decarboxylated and non-decarboxylated *C. sativa* strain CBD Charlotte's Angel female flower using ultrasound-assisted extraction (UAE) method with different solvents. Data are provided as a weight percentage of the extract obtained per 1 gram of dried cannabis flower.

Sample	% Yield
NH-Ethanol	16.61 ± 1.41%
NH-Hexane	9.69 ± 0.79%
H110-Ethanol	12.88 ± 1.90%
H110-Hexane	11.40 ± 0.27%

Table 4. Yield production of *C. sativa* obtained from ONCB Thailand using cold-maceration (conventional method) with different solvents. Data are provided as a weight percentage of the extract obtained per 5 grams for organic solvent extraction and 60 grams for SFE of dried cannabis materials.

Sample	% Yield
Ethanol	12.13 ± 1.54%
Hexane	10.38 ± 0.74%
Light Petroleum	7.60 ± 1.32%
Heavy Petroleum	10.39 ± 0.88%
SFE ($SCCO_2$)	1.92 ± 0.29%

The process of decarboxylation of the cannabis powder by heating the material before extraction also shows an effect with a difference in the percentage of yield produced from both organic solvents used in this study. Non-decarboxylation cannabis extracted using ethanol showed the highest yield compared to decarboxylated-cannabis which tended to be lower in yield production. The yield production from decarboxylated-cannabis extracted using hexane was higher than that which was not decarboxylated before extraction. Tallon et al (2018) also

obtained a similar result which shows decarboxylated cannabis produced a higher yield than non-decarboxylated cannabis especially used non-polar solvent due to decarboxylated form in the cannabis being more soluble in the non-polar solvent than acid form (Tallon et al., 2018).

The yield production results presented in Table 4 prove that ethanol consistently provides the best quality in terms of yield production compared to another organic solvent used in this study due to its polarity which is suitable for cannabis extraction. Indirectly, Table 3 and Table 4 show the yield production of cannabis depending on several factors such as solvent type, extraction method, and decarboxylation process.

4.2 Chemical Profiling

Nowadays, High-Performance Thin Layer Chromatography (HPTLC) become one of the new technologies that are a new generation of TLC which is very possible for identification, screening, and isolation of various active compounds in the plant by presenting data that has high resolution in terms of visualization. It also provides more accurate data and is globally accepted as one of the most powerful analytical techniques applied for phytochemical analysis (Bhargava et al., 2021; Sisodiya & Shrivastava, 2017). The data obtained from HPTLC analysis can be in form of qualitative data that provide information about chemical profile obtained from the separation of active compounds and also quantitative that provide information about quantification of the compound of interest which in this section we focused on qualitative data used to compare the chemical profile of cannabis extracted using both solvent groups.

HPTLC plate was developed by using mobile phase including heptane, diethyl ether, and formic acid with ratio 90:20:0.3 (v/v/v/). Cannabinoids standards such as CBG, CBDA, THCA, CBN, THC, and CBD have also been applied on the HPTLC plate

altogether with all the samples of organic solvent group extract and vegetable oil group extract. The development conditions for the HPTLC plate were modified according to the American Herbal Pharmacopoeia for chemical profiling of *C. sativa* (Pharmacopoeia, 2014)

4.2.1 The Influence of Decarboxylation Process on Chemical Profile of *C. sativa* strain CBD Charlotte's Angel Female Flower Extract

The influence of decarboxylation on the presence of cannabinoid acids and neutral cannabinoids of *C. sativa* was evaluated by using female flower extracts from *C. sativa* strain CBD Charlotte's Angel as plant materials which are extracted using organic solvent (ethanol & hexane) and vegetable oils (VCO and olive oil). The presence of each compound was detected through a chemical profile resulting from the separation of the compounds contained in the extract using the HPTLC method. The chemical profile formed on the HPTLC plate shows all the active constituents contained in the extract that was successfully separated using a predetermined mobile phase in the HPTLC instrument. After the separation process and scanning at UV_{254}, UV_{366}, white light with combined post-chromatographic derivatization using 1% vanillin reagent, the chemical profile of each extract is presented in Figure 12. White light after derivatization showed the best visualization of the chemical profile.

Cannabinoids such as CBD and CBDA in all tested samples were detected very strongly compared to other cannabinoids which were visually undetectable at R_f 0.35-0.50. The color of the CBD and CBDA bands that appear in each extract on the plate under white light looks slightly different from the respective standard due to the high concentration of both compounds contained in the extract resulting in a darker or more concentrated band color. On the plate scanned UV_{366} after derivatization shows very clear the presence of an unidentified band which was detected in all tested samples especially in samples extracted using olive oil. Those

compounds are also clearly on the plate scanned with visible light (white light). Several unidentified bands were also detected (R_f 0.1 – 0.3) which indicated the presence of other compounds contained in the extract on the plate scanned under UV_{366} and white light (after sprayed using 1% vanillin reagent) with various band strength which in samples extracted using vegetable oils showed strong bands compared to samples extracted using organic solvents. Several visible bands separated at R_f < 0.1 were detected on the plate scanned under UV_{366} and white light.

The presence of CBD and CBDA bands with varying band strengths in each sample tested proved that the decarboxylation process highly affects the presence of both compounds qualitatively. All the decarboxylated samples showed the strongest CBD bands than CBDA bands which indicated that the CBDA compound in the sample had been converted to accelerated to CBD that supported by heating the powder before extraction at 110°C for 60 minutes while all the non-decarboxylated samples showed the strongest CBDA bands than CBD bands which indicated that CBD compound was still concentrated in the acid form which is CBDA. Naturally, CBD and other neutral cannabinoids are presented as their corresponding carboxylic acid in cannabis (Kinghorn et al., 2017). Those neutral compounds are not formed in abundant concentrations but will be converted significantly through non-enzymatic decarboxylation processes when exposed to light or heat (Tan et al., 2018). The conversion of CBDA to CBD can be depicted in Figure 11.

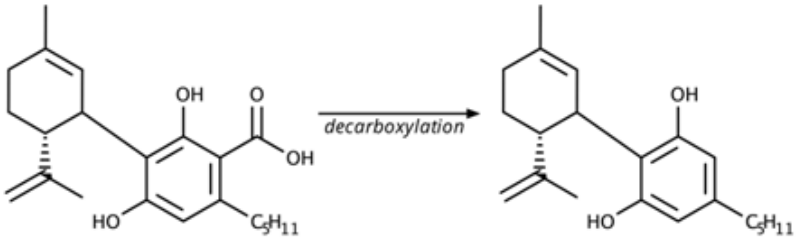


Figure 11. The conversion of CBDA to CBD

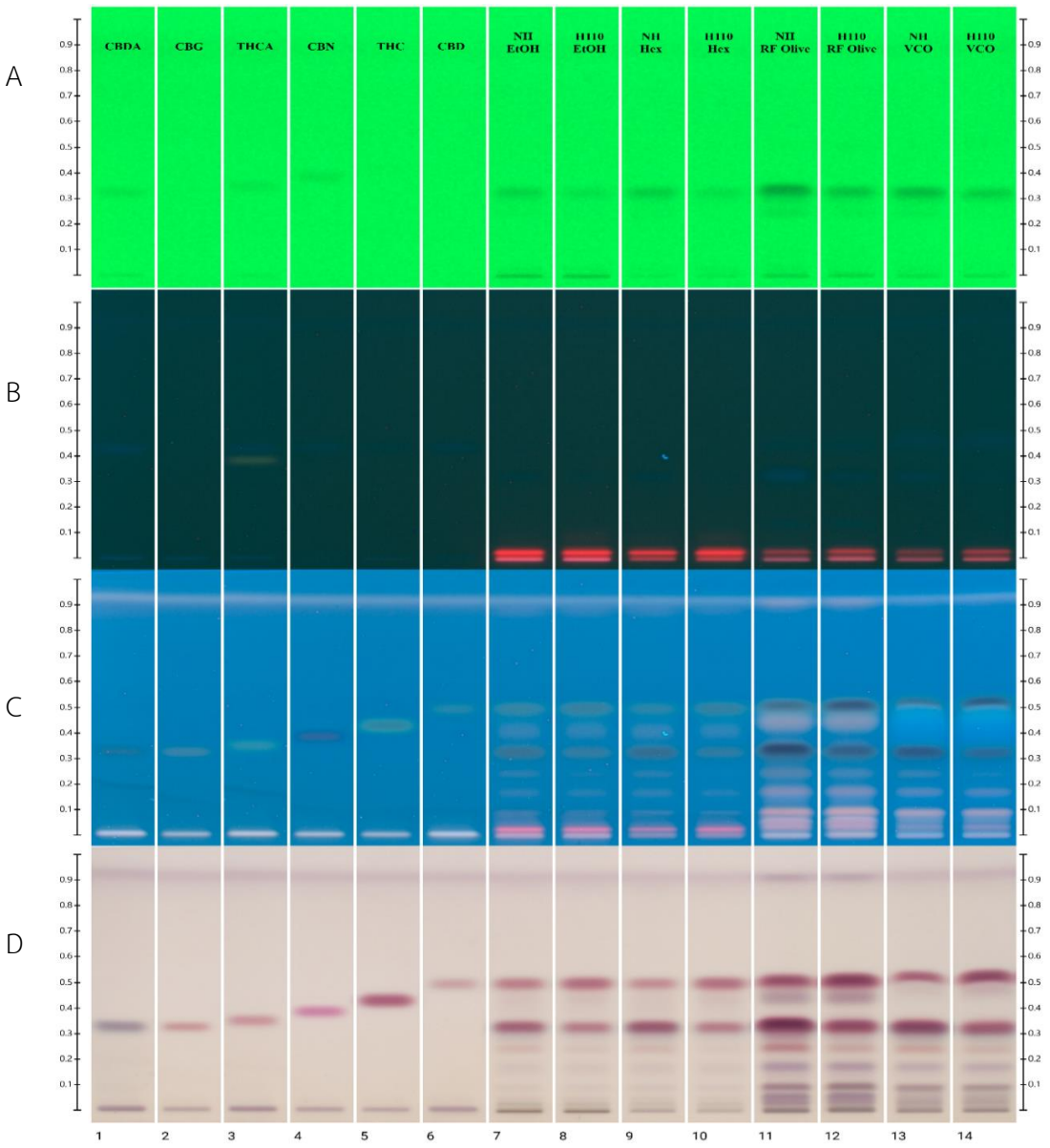


Figure 12. HPTLC Chemical Profile of *C. sativa* strain CBD Charlotte's Angel under UV_{254} (A); UV_{366} (B); UV_{366} (sprayed vanillin reagent) (C); White Light (sprayed vanillin reagent) (D). Track 1– CBDA; Track 2-CBG; Track 3-THCA; Track 4-CBN; Track 5-THC; Track 6-CBD; Track 7-NH Ethanol; Track 8-H110 Ethanol; Track 9-NH Hexane; Track 10-H110 Hexane; Track 11-NH RF Olive Oil; Track 12-H110 RF Olive Oil; Track 13-NH VCO; Track 14-H110 VCO.

4.2.2 The Influence of Solvent Types on Chemical Profile of *C. sativa*

The influence of solvent types on the chemical profile of *C. sativa* L. was examined using decarboxylated cannabis material obtained from ONCB Thailand. Cannabis material which used in this experiment is adulterated-cannabis flower that grounded into the powder then extracted using various organic solvents including ethanol, hexane, light petroleum, and heavy petroleum. Vegetable oils are used for extraction including VCO, olive oil, perilla seed oil, rice bran oil, sesame seed oil, and sacha inchi oil. Cannabis extracts including supercritical CO_2 and winterized supercritical CO_2 which were obtained from the supercritical fluid extraction method by using CO_2 as a solvent also included in this experiment. The material has been decarboxylated at 110°C for 60 minutes then soaked in the solvent overnight. After that, all samples and cannabinoids standards were applied to the HPTLC plate then developed using the same system as the previous experiment. The chemical profile after scanning at UV_{254}, UV_{366}, white light with combined post-chromatographic derivatization using 1% vanillin reagent is presented in Figure 12. White light after derivatization showed the best visualization of the chemical profile.

HPTLC results showed that the chemical profile of *C. sativa* L. extracted using various organic solvents, supercritical fluid extract, and various vegetable oils are look similar which was indicated by the presence of several cannabinoids such as CBG (dark orange), CBN (rosette), THC (brown), and also CBD (dark red) on the top and other unidentified compounds contained in the extract on the bottom. The presence of compounds detected as CBG, CBN, THC, and CBD was due to having the same R_f value (0.35 – 0.5) with cannabinoid standards and other unidentified compounds which detected that having R_f value (0.1 – 0.3). Qualitatively, the organic solvent extracts showed strong bands of CBD and CBN followed by THC and CBG. While vegetable oil extracts and supercritical CO_2 showed strong bands of CBD and CBN followed by THC and CBG. The chemical composition of a plant extract is greatly determined by the selection of solvent types as well as the extraction method used,

therefore the polarity of the compound's target is the crucial thing to be known beforehand (Azmir et al., 2013).

The similarity of the chemical profile of cannabis extracted using various organic solvents and vegetable oils indicated that vegetable oil has a good ability to dissolve cannabinoids. According to Pierre et al (2002) that solvents with low viscosity tend to be associated with good migration ability through the matrix, thereby increasing extraction efficiency (Pierre et al., 2002). Despite both groups of solvent, organic solvents (0.3 – 1.1 cP) and vegetable oils (35-90 cP) used in this study had significant differences in viscosity values, qualitatively vegetable oils are still able to dissolve several main cannabinoids in cannabis such as CBG, CBN, THC, and CBD as evidenced by the presence of these compounds in the chemical profile on all samples extracted using vegetable oils (Benítez et al., 2018; Diamante & Lan, 2014; Lee et al., 2021; Mohammed et al., 2021; Nierat et al., 2014; Siddiqui & Ahmad, 2013). Due to chemical structure, vegetable oil has long fatty acid chains that contain carbon-hydrogen bone which essentially has no dipole moment therefore vegetable oil is non-polar and considered suitable with cannabinoids which are also known chemically as fat-soluble and grouped into non-polar compounds.

Vegetable oils have been known as a solvent for extraction since ancient civilizations such as Egyptians, Phoenicians, Indians, and Chinese (Olson, 2010; Pitts et al., 2007). Currently, the good ability of vegetable oil as a solvent also has been proved by several studies. Chen & Meyers (1982) in their study used soy oil for astaxanthin pigment extraction from Crawfish waste (Chen & Meyers, 1982). A similar result was obtained by Handayani et al (2008) and Kang & Sim (2008) which extracted astaxanthin pigment from *Penaeus monodon* and Haematococcus (Handayani et al., 2008; Kang & Sim, 2008). Astaxanthin pigment also can be extracted from shrimp by using flaxseed oil (Pu et al., 2010). Li et al (2014) succeeded in proving experimentally and theoretically the good solubility of various vegetable oils in extracting volatile compounds (Li et al., 2014).

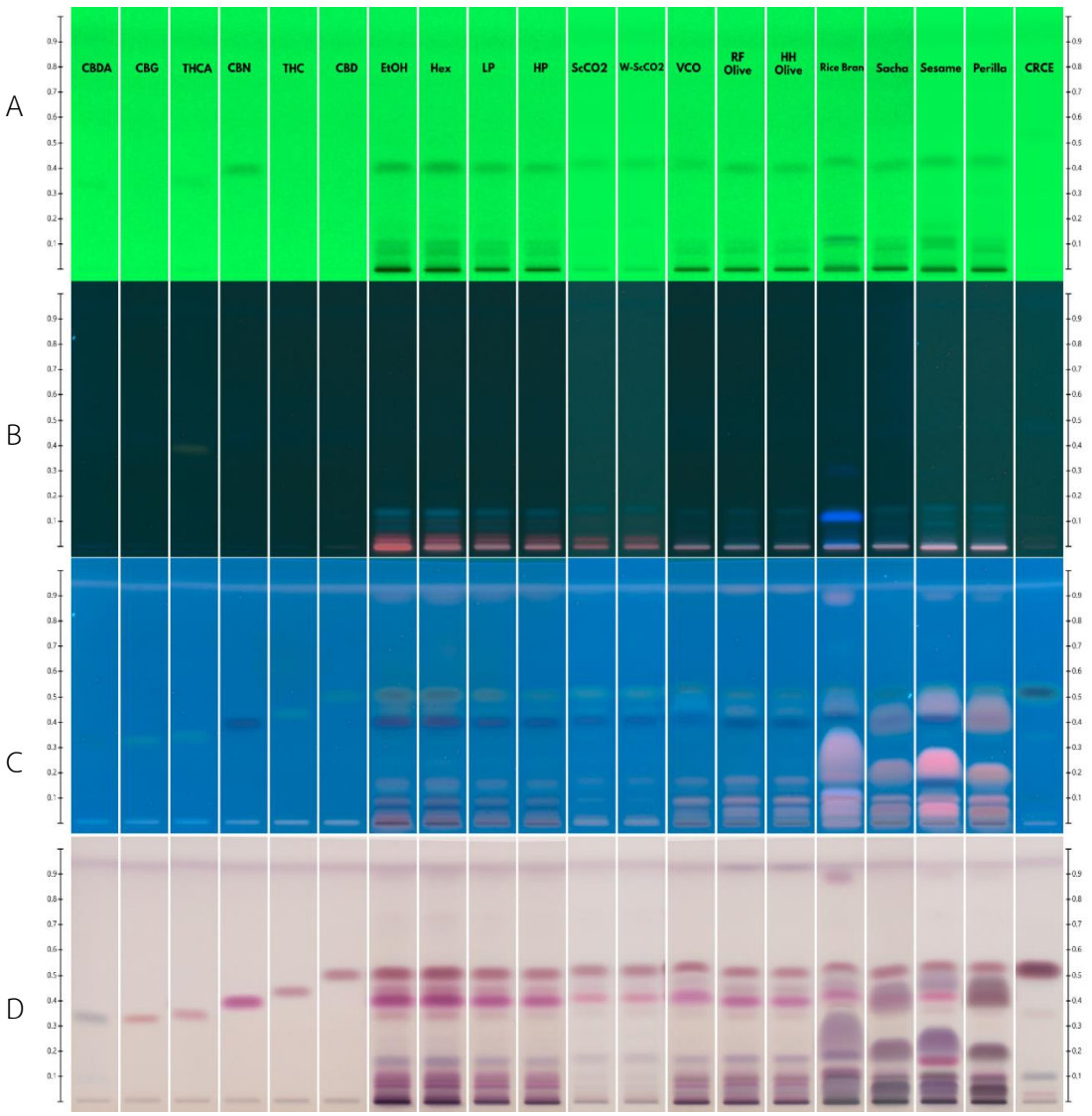


Figure 13. HPTLC Chemical Profile of *C. sativa* under UV_{254} (A); UV_{366} (B); UV_{366} (sprayed vanillin reagent) (C); White Light (sprayed vanillin reagent) (D). Track 1–CBDA; Track 2-CBG; Track 3-THCA; Track 4-CBN; Track 5-THC; Track 6-CBD; Track 7-Ethanol; Track 8-Hexane; Track 9-Light Petroleum; Track 10-Heavy Petroleum; Track 11-Supercritical CO2; Track 12-Winterized Supercritical CO2; Track 13-VCO; Track 14-RF Olive Oil; Track 15-HH Olive Oil; Track 16-.Rice Bran Oil; Track 17-Sacha Oil; Track 18-Sesame Oil; Track 19-Perilla Oil; Track 20-CBD Distillate (CRCE).

4.3 HPLC Quantification of Cannabinoids

Quantification of cannabinoids content was analyzed by using HPLC to confirm the effect of the decarboxylation process before extraction and the type of solvent used in the extraction process quantitatively. THC and CBD were chosen to quantify due to both compounds as major cannabinoids in *C. sativa*.

4.3.1 The Influence of Decarboxylation Process on the Presence of Cannabinoids

The quantification result of CBD and its acids form of *C. sativa* strain CBD-Charlotte's Angel female flower which was extracted using ethanol, hexane, virgin coconut oil, and olive oil is presented in Table 5 as well as statistical analysis of the result of CBD quantification is presented in Figure 14.

Table 5. Quantification of cannabinoid acid & neutral cannabinoid of *C. sativa* strain CBD-Charlotte's Angel female flower extract.

Samples	$CBDA_{(\%, w/v)}$	$CBD_{(\%, w/v)}$
	Organic Solvents	
NH-Ethanol	12.49 ± 2.59%	10.24 ± 1.74%
H110-Ethanol	12.00 ± 2.22%	15.37 ± 3.54%
NH-Hexane	18.96 ± 0.65%	4.86 ± 0.31%
H110-Hexane	10.92 ± 0.38%	17.71 ± 0.20%
	Vegetable Oils	
NH-VCO	0.39 ± 0.12%	0.12 ± 0.03%
H110-VCO	0.39 ± 0.14%	0.36 ± 0.15%
NH-Olive Oil	0.31 ± 0.21%	0.18 ± 0.05%
H110-Olive Oil	0.31 ± 0.10%	0.17 ± 0.04%

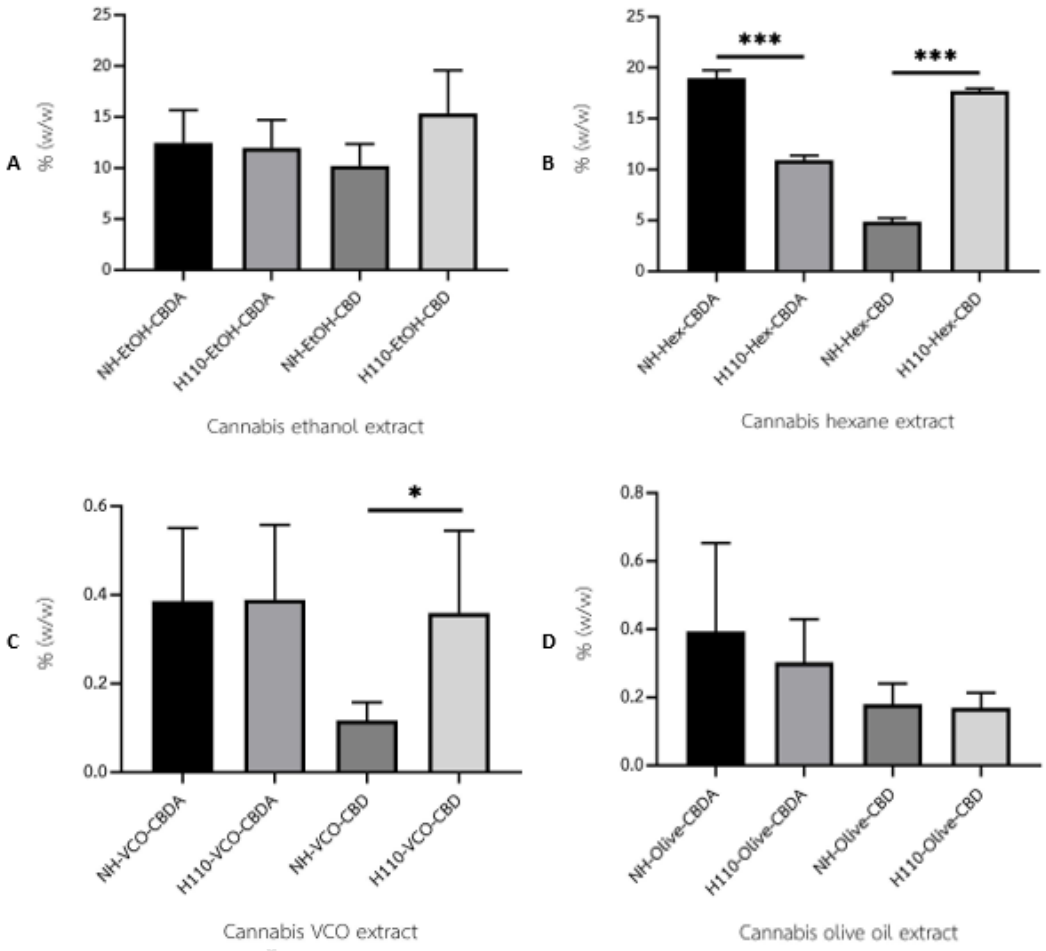


Figure 14. Quantification of CBDA and CBD of *C. sativa* extracts, (A) Ethanol extract; (B) Hexane extract; (C) Virgin Coconut Oil extract; (D) Roasting & Frying Grade of Olive Oil. All the tested sample was divided into 2 groups: NH (Non-decarboxylated) and H110 (Decarboxylated at 110ºC for 60 minutes before extraction). All the experiment was in triplicate (n=3) and analyzed statistically by using non-parametric (t-test) which (*) means significantly different (p<0.05).

The decarboxylation process can be accelerated by heating the material before or during the extraction process. In this study, we decarboxylated all the

samples before extraction and analyze the THC and CBD in respective samples. Chemically, the main cannabinoid presence in acids form (Martinenghi et al., 2020). These results have confirmed the change of cannabinoid acids such as CBDA into neutral cannabinoids such as CBD quantitatively. CBDA was detected in abundance in all organic solvents extract that was not decarboxylated before extraction while CBD was detected to be lower than CBDA. Statistically, hexane extract which is decarboxylated and non-decarboxylated extract showed significant differences ($p < 0.05$) in the number of CBDA and CBD. Likewise, VCO also showed significant differences ($p < 0.05$) in terms of the number of CBD. The result of quantification of CBD and CBDA content showed variation in the ratio of both compounds. CBDA was exhibited higher in all organic solvent extracts that were not decarboxylated while CBD was detected higher in decarboxylated organic solvent extracts. The ethanol extract which was not heated before extraction showed the CBDA content of 12.49 ± 2.59% while hexane extract was 18.96% ± 0.65%. When the heating was carried out before extraction at 110°C for 60 minutes, CBDA in both extracts decreased to 12.00 ± 2.22% and 10.92 ± 0.38% respectively. It is due to cannabinoid acid which is CBDA in the extract have been decarboxylated into neutral cannabinoids therefore it can be seen in the CBD content which increased from 10.24 ± 1.74% to 15.37 ± 3.54% in ethanol extract and 4.86 ± 0.31% to 17.71 ± 0.20% in hexane extract.

Likewise, CBDA content in virgin coconut oil extract and olive oil did not decrease after being heated at 110°C which CBDA content of 0.39 ± 0.12% and 0.31 ± 0.21% respectively while an increase occurred in the content of CBD in the heated sample which from 0.12 ± 0.03% contained in the unheated sample to 0.36 ± 0.15%. Surprisingly, CBD content showed a slight decrease in the heated sample of olive oil extract. The HPLC chromatogram result also confirmed the chemical profile of extract by the presence of CBDA and CBD in the respective organic solvent extract and vegetable oil extract (see Appendix pages 101-104). The presence of CBDA in decarboxylated samples indicates an incomplete decarboxylation process. This could

be due to the heating temperature and time which are parameters of this process (Nuapia et al., 2021) that do not make the decarboxylation process run efficiently. Preheating the plant material at 110°C for 60 minutes could not complete this process which was confirmed by the presence of CBDA in the decarboxylated extract despite the CBD showing an increase in the decarboxylated samples. In addition, THCA and THC in the extract were detected to be in very low amounts and even undetectable in several extracts due to the cannabis strain that used was as CBD-rich cannabis. Therefore, it is inexecutable to do a comparison and evaluation of the effect of pre-heating on the presence of both compounds in respective extracts quantitatively.

4.3.2 The Influence of Solvent Types on the Cannabinoids Content

As discussed in the previous discussion where the selection of solvent type for extraction will greatly affect the yield production and chemical profile of *C. sativa* The selection of solvent type is an urgent issue in terms of working on plant raw material which will assure the release of the active constituents from the matrix maximally (Shakun et al., 2020).

Plant material which is cannabis that has been extracted using common organic solvents for cannabis extraction including ethanol, hexane, petroleum ether, and supercritical CO2 was analyzed for the THC and CBD content using HPLC in the respective extract as well as vegetable oils including virgin coconut oil, olive oil, perilla seed oil, sacha inchi oil, rice bran oil, and sesame seed oil which were also used as a solvent. The result of HPLC analysis of the content of THC and CBD in the cannabis extract is presented in Table 6 and then the data is statistically analyzed using ANOVA one-way and non-parametric test (t-test) which is presented in Figure 15-16.

Table 6. Quantification of major cannabinoids of *C. sativa* obtained from ONCB Thailand.

Samples	$CBD_{(\%,w/w)}$	$THC_{(\%,w/w)}$
Ethanol	4.43 ± 0.44%	1.34 ± 0.22%
Hexane	3.77 ± 1.41%	0.71 ± 0.40%
Light Petroleum	4.82 ± 0.15%	0.59 ± 0.30%
Heavy Petroleum	4.36 ± 0.39%	0.39 ± 0.32%
Supercritical CO_2	2.60 ± 0.06%	7.20 ± 0.055%
Winterized Supercritical CO_2	3.89 ± 0.14%	13.29 ± 0.02%
Virgin Coconut Oil	0.01 ± 0.004%	0.03 ± 0.01%
RF Olive Oil	0.01 ± 0.000%	0.03 ± 0.0005%
HH Olive Oil	0.01 ± 0.002%	0.03 ± 0.0024%
Sacha Inchi Oil	0.02 ± 0.004%	0.03 ± 0.005%
Sesame Seed Oil	0.01 ± 0.002%	0.02 ± 0.003%
Perilla Seed Oil	0.01 ± 0.003%	0.02 ± 0.004%
Rice Bran Oil	0.01 ± 0.003%	0.03 ± 0.004%

Remarks: RF (roasting & frying grade); HH (high-heat cooking grade)

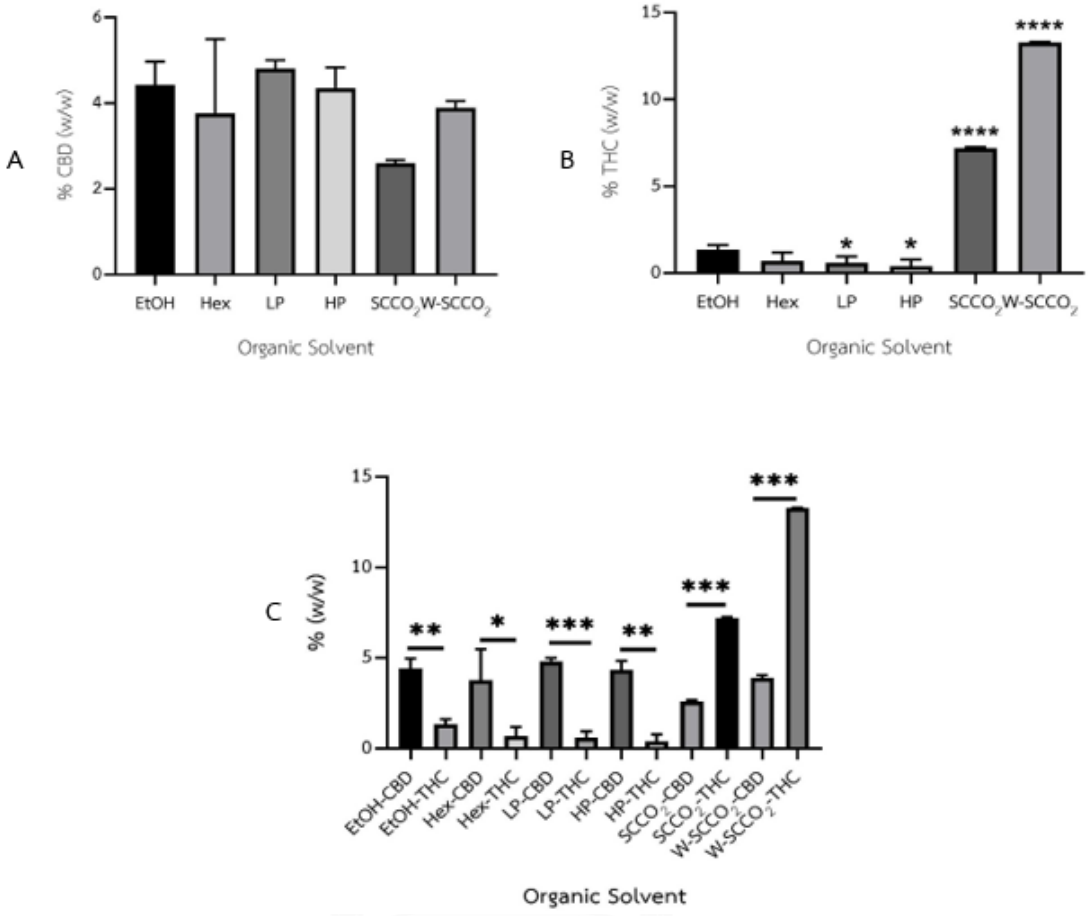


Figure 15. Quantification of THC and CBD of *C. sativa* organic solvent extracts, (A) THC content of organic solvent extract (%, w/w); (B) CBD Content of organic solvent extract; (C) Comparison of THC and CBD content of the organic solvent extract. All the experiment was in triplicate (n=3) and analyzed statistically by using non-parametric (t-test) and ANOVA one-way (ethanol extract as a standard). (*) means significantly different (p<0.05).

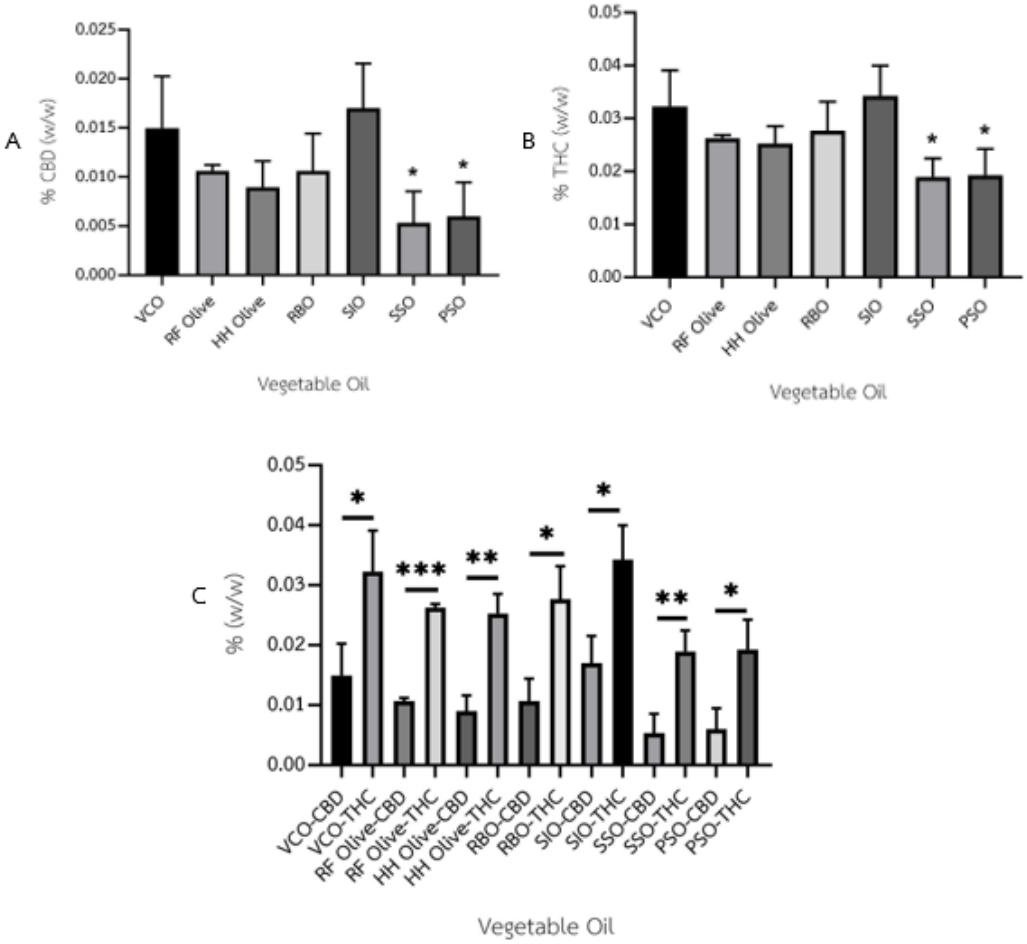


Figure 16. Quantification of THC and CBD of *C. sativa* vegetable oil extracts, (A) THC content of vegetable oil extracts (%, w/w); (B) CBD content of vegetable oil extracts (%, w/w); (C) Comparison of THC and CBD content of vegetable oil extracts. All the experiment was in triplicate (n=3) and analyzed statistically by using non-parametric (t-test) and ANOVA one-way (ethanol extract as a standard). (*) means significantly different (p<0.05).

The solvent ability in dissolving compounds from the material greatly determines the number of active compounds in the plant extract. HPLC

Chromatogram confirmed that THC and CBD were detected in all extracts (see Appendix pages 105-108). The result of the HPLC analysis of THC and CBD showed that petroleum ether with a low boiling point (light petroleum) could extract the CBD with the highest content in the organic solvent group which was 4.82 ± 0.15% and followed by ethanol extract 4.43 ± 0.44%. While in the vegetable oil group, sacha inchi oil showed the highest CBD content of 0.02 ± 0.004% which the rest of the vegetable oil contained an equal CBD content. Likewise, supercritical CO_2 and winterized supercritical CO_2 showed the highest content of THC which was 13.29 ± 0.02% and 7.20 ± 0.055% respectively in the organic solvent group while the vegetable oil group had the same amount of THC in the range of 0.02-0.03%. Statistically, there was a significant difference in the ratio of THC and CBD content of the respective extract where ethanol, hexane, petroleum ether extract showed a high content of CBD compared to THC, in contrast to the supercritical CO_2 and vegetable oils group which showed a high content of THC content compared to CBD. The amount of CBD in the organic solvent group showed insignificant different while in vegetable oil group showed sesame seed oil and perilla seed oil have CBD content which is significantly different ($p<0.05$) from virgin coconut oil as a standard. Supercritical CO_2 and winterized supercritical CO_2 extract showed a very significant difference ($p<0.05$) in THC content to ethanol extract while perilla seed oil extract, as well as sesame seed oil extract, also showed a significant difference ($p<0.05$) in THC content to virgin coconut oil extract.

As discussed previously, the solvent is a crucial factor in the extraction process where the solvent greatly determines the quality and quantity of the compounds target obtained from the extraction. The polarity of the compound of interest can be used as a reference for selecting the solvent to be used for the extraction. Ethanol, hexane, and petroleum ether are commonly used for cannabis extraction (Abubakar et al., 2020; Krill et al., 2020; Laznik et al., 2020). Supercritical Fluid Extraction (SFE) is a technology that is often applied in vegetable matrices to

extract essential oils and various active constituents on a large scale which CO_2 is commonly used for extraction (de Melo et al., 2014; Herrero et al., 2010; King, 2014; Reverchon & De Marco, 2006). This method provides several benefits such as ultra-pure quality and low solvent residue (Shinde & Mahadi, 2019). Thus, it correlates when compared to other solvents used in the experiment in terms of the amount of THC and CBD produced from this method is much higher. Currently, this method has been conducted by several studies (Baldino et al., 2020; Grijo et al., 2019; Rovetto & Aieta, 2017). Purification in supercritical CO_2 extraction or well-known as winterization which can be used for removing the wax, chlorophyll, and fat from the extract therefore the purity of cannabinoids and other compounds in cannabis extract also increased. It is proved by the increase of THC and CBD compared to supercritical CO_2 without winterization.

Since we did not vaporize the vegetable oil from pure extract, the viscosity of vegetable oil used for extraction is thought to be a major factor affecting the quality of cannabinoids extraction which can compare indirectly to organic solvents. Refers to literature which is summarized in the introduction section, vegetable oil used in this study has a high viscosity with a value range of 36.5 – 88 cP (Diamante & Lan, 2014; Ghani et al., 2018; Lee et al., 2021; Mai et al., 2020; Nierat et al., 2014; Siddiqui & Ahmad, 2013). The solvent with high viscosity can also be slackening the extraction process of active constituents contained in cannabis. Despite cannabinoids being known to be non-polar compounds and oil-soluble, high viscosity causes the penetration of vegetable oil into the matrix to be slower compared to organic solvent as well as maceration duration which is strongly suspected to be ineffective in optimizing the extraction process. Surprisingly, the viscosity of each vegetable oil did not affect the extracted cannabinoids content due to the content of both compounds contained in the respective vegetable oils did not differ much, namely, in the range of 0.01-0.02% (w/v). Therefore, the selection of the best vegetable oil in extracting cannabinoids is largely determined by its health benefit. As known,

vegetable oil has physicochemical properties which greatly affect human health if consumed. For healthy consumption, vegetable oils such as olive oil, perilla seed oil, sacha inchi oil, sesame seed oil, and rice bran oil are good choices for consumption altogether with cannabis extract. Unsaturated oil is considered to have good benefits for human health compared to saturated oil which can promote some health risks such as cardiovascular and coronary heart disease (Mozaffarian & Clarke, 2009).

4.4 The Influence of Solvent Types on Antioxidant Activity of *C. sativa*

In this study, the antioxidant activity of cannabis extract was evaluated using two methods, TLC-Bioautography, and colorimetric assay. The cannabis extract used in this experiment was cannabis materials from ONCB Thailand (adulterated-cannabis flower) which aims to evaluate the influence of solvent types on the antioxidant activity of cannabis.

4.4.1 Radical Scavenging Assay (DPPH Colorimetric) of *C. sativa* Extract

The antioxidant power of cannabis extract was examined colorimetrically using DPPH as a free radical. The decolorization that occurred after the addition of the respective extracts solution was measured for the optical density (OD) value using UV light with λ_{517}. The IC_{50} of the respective extract is presented in Table 9. Among all the organic solvent extracts, winterized supercritical CO_2 extract showed the strongest antioxidant power with IC_{50} of 0.03 ± 0.006 mg/mL followed by hexane extract (IC_{50} 0.05 ± 0.003 mg/mL) and supercritical CO_2 (IC_{50} 0.07 ± 0.004 mg/mL). While for vegetable oils group, rice bran oil showed the strongest antioxidant power with an IC_{50} value of 3.35 ± 0.120 mg/mL) followed by virgin coconut oil (VCO) and sacha inchi oil with IC_{50} values of 5.81 ± 1.396 mg/mL and 7.05 ± 0.081 mg/mL respectively. The gap in IC_{50} values among both solvent groups was made possible by the ability of the solvent to dissolve the active constituents that contribute to

overall antioxidant power in the plant material which has also been proven by the significantly different content of THC and CBD in both groups. In addition, IC_{50} values in all samples extracted using organic solvents were not significantly different, while in vegetable oil are significantly different in the IC_{50} values range of 3.35-13.70 mg/mL.

Since the vegetable oil used to extract the material in this study was not evaporated and separated from the pure extract, the overall antioxidant power of vegetable oils samples is also strongly suspected as a contribution from the chemical component of vegetable oils itself which is known to have fatty acid as a major component. The proportion and types of fatty acids that make up vegetable oils vary widely. Several studies reported the proportion of fatty acid of various vegetable oils as well as minor components in vegetable oils which are also known to have antioxidant activity (Asif, 2011; Cisneros et al., 2014; Dachtler et al., 2003; Dossa et al., 2017; Ghani et al., 2018; Kang et al., 1998; Kizhiyedhatu Polachira et al., 2004; Lee et al., 2008; Mohammed et al., 2021; Silenzi et al., 2020; Suja et al., 2005; Xiang et al., 2017). Tocopherol is one of the minor components commonly found in edible oils. Tocopherol promotes oxidative stability of vegetable oil which is one of the crucial properties of vegetable oil (Gliszczyńska-Świgło et al., 2007). Regarding the report by Chiba (1999), the fatty acid composition and carbonyl value are also considered as a determinant of oxidative stability of vegetable oil (Chiba, 1999).

Table 6. DPPH radical scavenging of organic solvents and vegetable oil extract of *C. sativa* All the experiment was done in triplicate (n=3) ± standard deviation. The data are sorted based on the lowest IC_{50} value which is interpreted as having the strongest antioxidant power against DPPH in each solvent group.

Samples/Standard	IC_{50} Value (mg/mL)
Gallic Acid	0.008 ± 0.0005
Organic Solvents	
W-$ScCO_2$	0.03 ± 0.006
Hexane	0.05 ± 0.003
$ScCO_2$	0.07 ± 0.004
Ethanol	0.08 ± 0.039
Light Petroleum	0.14 ± 0.017
Heavy Petroleum	0.35 ± 0.022
Vegetable Oils	
Rice Bran Oil	3.35 ± 0.120
Virgin Coconut Oil	5.81 ± 1.396
Sacha Inchi Oil	7.05 ± 0.081
Olive Oil (HH)	7.66 ± 0.753
Olive Oil (RF)	11.88 ± 0.375
Perilla Seed Oil	13.14 ± 1.062
Sesame Seed Oil	13.70 ± 0.565

4.4.2 TLC-Bioautography *C. sativa* Extract

Detection of antioxidant activity of cannabis extracts on DPPH free radicals was also carried out qualitatively using TLC-DPPH to confirm that the active constituents contained in the extract had antioxidant properties. All samples including cannabinoid standards that have been applied to the TLC plate are then

developed with the existing system. Furthermore, the chemical profile obtained from the separation of the compounds on the TLC plate was sprayed using 0.2% DPPH in methanol and incubated for 30 minutes in a dark room. The yellow color that appears on the purple background indicated compounds that have antioxidant activity. The TLC-Bioautography DPPH of cannabis extract is presented in Figure 17.

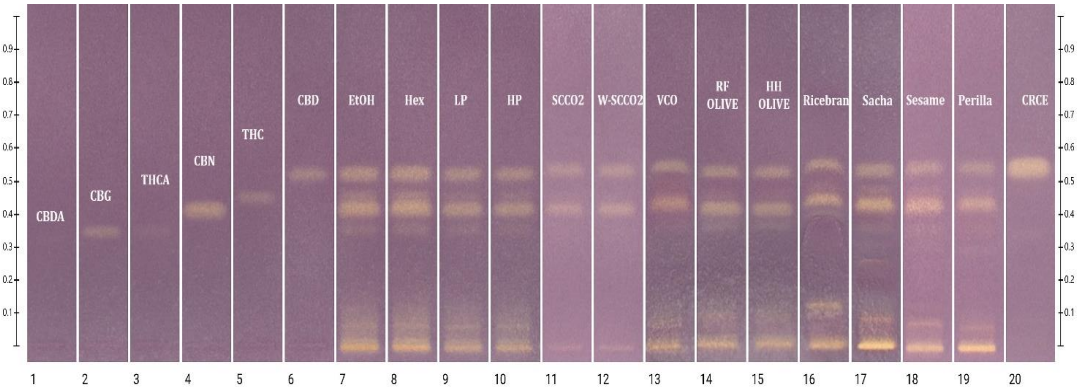


Figure 17. TLC Chemical Profile of *C. sativa* with post-chromatographic derivatization using 0.2% DPPH in Methanol. Track 1– CBDA; Track 2-CBG; Track 3-THCA; Track 4-CBN; Track 5-THC; Track 6-CBD; Track 7-Ethanol; Track 8-Hexane; Track 9-Light Petroleum; Track 10-Heavy Petroleum; Track 11-Supercritical CO2; Track 12-Winterized Supercritical CO2; Track 13-Virgin Coconut Oil; Track 14-Olive Oil Roasting & Frying Grade; Track 15- Olive Oil High Heat Cooking Grade; Track 16-Rice Bran Oil; Track 17-Sacha Inchi Oil; Track 18-Sesame Seed Oil; Track 19-Perilla Seed Oil; Track 20-CBD Distillate (CRCE).

Detection of antioxidant of cannabis extracts guided by TLC-Bioautography showed that several compounds separated on the TLC plate had antioxidant activity. CBD contained in all the samples has strong antioxidant properties against DPPH free radicals as indicated by the presence of yellow bands in respective samples with very strong intensity. A similar result was also seen in CBN compounds detected in

all samples which showed strong and uniform yellow band intensity as well as pure CBN as a standard. This indicates that CBN also has antioxidant properties. The THC compounds detected in all samples tested also showed antioxidant activity. However, it is not as strong as CBD and CBN where the intensity of the yellow band in all samples is weak and slightly strong in ethanol and hexane extract. Several unidentified bands also appear to have antioxidant properties against DPPH free radicals which are indicated by the appearance of yellow bands at the bottom zone. These results also confirmed from the previous experiment that the overall antioxidant activity of cannabis extract was not only contributed by cannabinoids but also other compounds. As it is known that plant extracts have a complexity of active compounds which might have antioxidant properties. Especially in the oils group where vegetable oils used as solvent also contain several minor components to have antioxidant properties.

Cannabinoids as our focus in this study are known to have antioxidant activity including CBG, CBDA, THCA, CBN, THC, and CBD which has been proven by Dawidowicz et al (2021) in which those compounds have been shown to scavenge free radicals (Dawidowicz et al., 2021). Regarding several studies of the literature revealed that THC has similar properties as phenolics in terms of its antioxidant properties, in which THC loses one electron and proton as an unpaired balancer (Balbino et al., 2012; Nissim & Compton, 2015; Novak et al., 2013; Smith et al., 2015). Hampson et al (1998) demonstrated the antioxidant properties of CBD and THC through cyclic voltammetry (Hampson et al., 1998). As mentioned, non-cannabinoids in cannabis also have an antioxidant activity such as polyphenol, and terpenes in cannabis are also known to scavenge free radicals (Cantele et al., 2020).

4.5 Cytotoxicity & UVA-Photoprotective Effect of *C. sativa* Extract on Human Skin Keratinocytes (HaCat Cells)

Human skin keratinocytes cell that has been cultured and incubated under predetermined conditions were then treated using cannabis extracts including ethanol extract, supercritical CO_2, winterized supercritical CO_2, and virgin coconut oil. Cannabinoid standards such as THC and CBD are also included in this experiment. Cells treated with cannabis extracts, THC, and CBD were also performed firstly to determine the cytotoxicity of all samples tested before further treatment using UVA radiation. The results of cytotoxicity of cannabis extracts, THC, and CBD are shown in Figure 18. Pure cannabinoids such as THC and CBD showed a non-toxic reaction to the cell in the concentration range 155-1250 ng/mL after being added to cell culture, which was characterized by the consistency of cell viability at 100%, although slightly increased at concentration 1250 ng/mL in CBD and 310 ng/mL in THC. Cannabis extracted using ethanol and supercritical CO2 showed a very significant increase in cell viability at the concentration range of 155-1250 ng/mL. It is indicated that these three samples are non-toxic to HaCat cells in that concentration range. Meanwhile, cannabis extracted using virgin coconut oil also showed non-toxic on HaCat cells.

Cytotoxicity test is the evaluation to observe growth cells and morphological effect biologically using tissue cell in vitro which is preferred as indicator toxicity of the compound which the success in the development of the pharmaceutical products is supported by minimal toxicity of the compound to the cells (Li et al., 2015; Yonbawi et al., 2021). Human skin keratinocytes or well-known as HaCat cells are mostly used as an epidermal model due to their presence on the epidermis (90%) and are most frequently used as in-vitro research for preliminary toxicity (Poumay & Coquette, 2007; Seo et al., 2012). In this study, testing the toxicity of the extract also provides information on the suitable concentration range (non-toxic) of the extract/compound for further experiment. Determination of cell viability was analyzed by using MTT (dimethylthiazol-diphenyltetrazolium bromide) assay.

This method is used to determine the mitochondrial status of the cells functionally. Living cells will produce the mitochondrial dehydrogenase enzyme which reduces yellow MTT tetrazolium salt (yellow) to MTT formazan blue (Edmondson et al., 1988).

The viability of cell which is stable and tends to increase significantly after 18 hours of incubation indicated that cannabis extract was tested on HaCat cells with the concentration range (THC equivalent) of 155-1250 ng/mL for ethanolic, supercritical CO_2, winterized supercritical CO_2 and CBD (155-1250 ng/mL), as well as VCO extract (THC equivalent; 6.75-54 ng/mL), is safe for HaCat cells especially in ethanolic extract, supercritical CO_2 and winterized supercritical CO_2 where the cell viability continued to increased (dose-dependent manner) for 18 hours treated with these extract. The cause of enhancement in cell viability of cannabis extract-treated HaCat cells has yet to be determined.

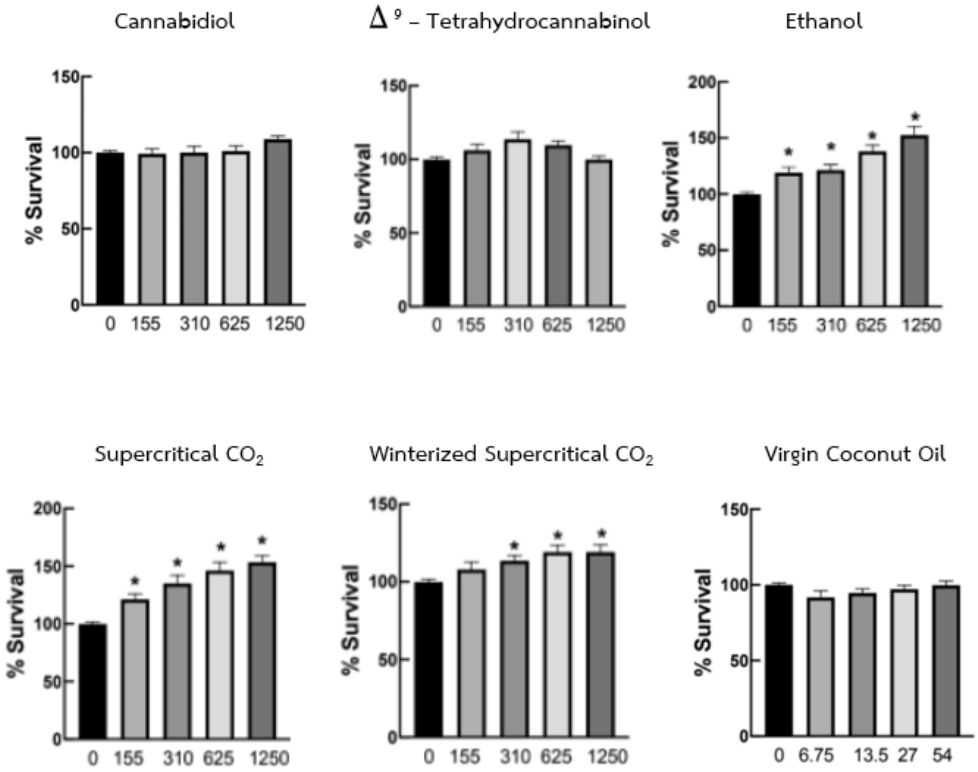


Figure 18. Cytotoxicity activity of *C. sativa* extracts on human skin keratinocytes (HaCat cells). HaCat cells were treated with THC, CBD, ethanolic extract, supercritical CO_2 extract, and winterized supercritical CO_2 extract (0-1250 ng/mL) and VCO extract (0-54 ng/mL) for 18 hours (n=3). The viability of HaCat cells after being treated using samples tested was expressed in the percentage of survival. (*) means significant difference with the control ($p < 0.05$).

Cannabis extract-treated HaCat cells for photoprotection assay were then exposed to UVA radiation (365 nm; 7 J/cm^2). Cell viability was then analyzed by using an MTT assay. The viability of UVA-Irradiated HaCat cells is presented in Figure 19. The result showed that cannabinoid-treated HaCat cells (pure THC and CBD) which were then irradiated with UVA did not protect HaCat cells effectively. It is characterized by insignificant differences in cell viability between treated cells and untreated cells (control). Interestingly, cannabis extract-treated cells including

ethanolic extract, supercritical CO2, and winterized supercritical CO2 showed a significant difference with the control in cell viability after exposure to UVA which indicated that these extracts can protect the UVA-Irradiated HaCat cells effectively. While VCO-treated cells also showed an insignificant difference in cell viability with the control.

Ultraviolet is one of the physical factors that can cause several problems, especially to human skin cells due to excessive radiation. Inflammation, cell proliferation inhibition even cell death are several harmful of excessive ultraviolet radiation (Panich et al., 2016). To the best of our knowledge, this is the first study to aim for evaluating the photoprotective effect of cannabis extracts on human skin keratinocytes. The result showed that cannabis extracts such as ethanolic, supercritical CO2, and winterized supercritical CO2 increased the viability of HaCat cells. This effect connects to our previous experiment which detected cannabinoids including CBG, CBN, THC, and CBD in those extracts that have antioxidant activity on DPPH free radicals. According to literature, several modes of UV photoprotection by natural antioxidants from the plant are modulate the antioxidant innate and stimulates the inflammation response (Saewan & Jimtaisong, 2015).

Pure cannabinoids used in this study including THC and CBD showed the photoprotective effect on UVA-Irradiated HaCat cells insignificantly different from untreated-HaCat cells compared to cannabis extract-treated such as ethanolic, supercritIcal CO_2, and winterized supercritical CO_2 which showed a significant difference from untreated-HaCat cells (control). Therefore, from our findings above can be concluded that the photoprotection ability of UVA-Irradiated HaCat cells from cannabis extracts such as ethanolic extract, supercritical CO_2 extract, and winterized supercritical CO_2 extract is also contributed by other cannabinoid compounds and non-cannabinoids contained in the extract that was not analyzed in this study. The combination of multiple compounds may greatly affect the overall UV photoprotection power of cannabis extracts. It is also supported by the results of the

antioxidant evaluation of cannabis extracts which showed supercritical CO_2 extract has the highest IC_{50} value followed by the ethanolic extract. Polyphenols such as flavonoids and phenols isolated from *C. sativa* were also reported to have antioxidant properties (Drinic et al., 2018). An insignificant result of virgin coconut oil extract is due to the concentration of virgin coconut oil extract used being too low therefore ineffective in protecting HaCat cells from UVA radiation.

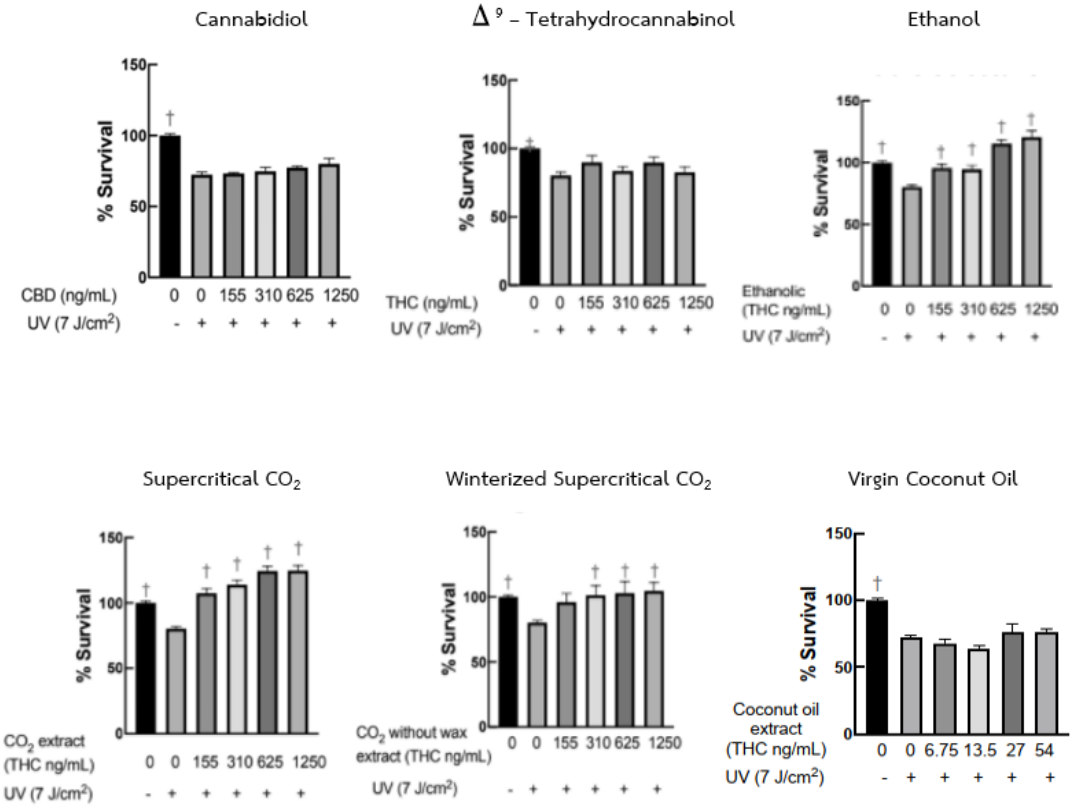


Figure 19. Photoprotective effect of *C. sativa* extracts on UVA-Irradiated HaCat cells). HaCat cells were treated with THC, CBD, Ethanolic extract, Supercritical CO_2 extract, and Winterized Supercritical CO_2 extract (0-1250 ng/mL) and VCO extract (0-54 ng/mL) (n=3). The viability of HaCat cells after being radiated by UVA was expressed in the percentage of survival. (†) means a significant difference with the control ($p < 0.05$).

CHAPTER V

CONCLUSION

Yield production in cannabis extraction is greatly affected by solvents used in the extraction process. Ethanolic extract of *C. sativa* is consistently to be a good solvent in terms of yield production compared to other organic solvents used in this study. A similar chemical profile of cannabis extracted using vegetable oils with organic solvents proved that vegetable oils can be used as an alternative solvent for cannabinoids extraction as well as decarboxylation process carried out before extraction also showed a similar chemical profile of flower of CBD Charlotte's Angel strain extracted using organic solvents and vegetable oils. The presence of CBDA and CBD with varying band intensities proved the decarboxylation reaction occurred in the extract after pre-heating the material. The solvent used also affects the quantity of THC and CBD. The presence of several cannabinoids such as CBG, CBN, THC, and CBD showed antioxidant activity against DPPH free radicals where winterized supercritical CO_2 extract (organic solvent) and RBO (vegetable oil) exhibit the strongest IC_{50} value oh DPPH free radicals in their group respectively. Other unidentified compounds also appeared and showed their antioxidant activity on the TLC plate. Ineffectiveness of pure THC and CBD in protecting HaCat cells from UVA radiation proved that the photoprotective effect of *C. sativa* not only contributed by THC and CBD as major cannabinoids but also other cannabinoids and non-cannabinoids contained in the extract which was not analyzed in this study. However, the ability of vegetable oils in dissolving cannabinoids such as CBD and THC was not as strong as organic solvents quantitatively. The high viscosity of vegetable oils reduces the effectiveness of the extraction process. The use of high temperatures during the extraction process which is known to reduce the viscosity of the vegetable oils needs to be considered for future studies.

REFERENCES

Aazza, S. (2021). Application of Multivariate Optimization for Phenolic Compounds and Antioxidants Extraction from Moroccan Cannabis sativa Waste. *Journal of Chemistry*, *2021*. https://doi.org/10.1155/2021/9738656

Abubakar, Y. U., Taura, D. W., Abdulhamed Baba Njobdi, P. O., & Ihedigbo, M. U. L. (2020). Antibacterial activity of extracts against Gallinarum Cannabis sativa Salmonella isolated from Poultry. *Advance Pharmaceutical Journal*, *5*(6), 183-189.

Adams, R., & Hunt, M. (1940). Structure of Cannabidiol, a Product Isolated from the Marihuana Extract of Minnesota Wild Hemp. I. *Journal of the American Chemical Society*, *62*(1), 196-200. https://doi.org/10.1021/ja01858a058

Aiello, A., Pizzolongo, F., Scognamiglio, G., Romano, A., Masi, P., & Romano, R. (2020). Effects of supercritical and liquid carbon dioxide extraction on hemp (Cannabis sativa L.) seed oil. *International Journal of Food Science and Technology*, *55*(6), 2472-2480. https://doi.org/10.1111/ijfs.14498

Akula, S., Nagaraja, A., Ravikanth, M., Raj Kumar, N., Kalyan, Y., & Divya, D. (2021). Antifungal efficacy of lauric acid and caprylic acid - Derivatives of virgin coconut oil against Candida albicans. *Biomedical and Biotechnology Research Journal*, *5*(2), 229-234. https://doi.org/10.4103/bbrj.bbrj_65_21

Alshahrani, S., Al Sreaya, A. A., Mashyakhi, M. Y., Alqahtani, S., Sivakumar, S. M., Alhazmi, H. A., Rehman, Z., & Alam, F. (2020). Chemical characterization and antibacterial efficacy of Saudi sesame oil against human pathogenic bacteria. *Environment Conservation Journal*, *21*(1&2), 19-29. https://doi.org/10.36953/ecj.2020.211203

Aluyor, E. O., Ozigagu, C. E., Oboh, O. I., & Aluyor, P. (2009). Chromatographic analysis of vegetable oils: A review. *Scientific Research and Essays*, *4*(4), 191-197.

Amaro-Ortiz, A., Yan, B., & D'Orazio, J. A. (2014). Ultraviolet radiation, aging and the skin: Prevention of damage by topical cAMP manipulation. *Molecules*, *19*(5), 6202-6219. https://doi.org/10.3390/molecules19056202

Andre, C. M., Hausman, J. F., & Guerriero, G. (2016). Cannabis sativa: The plant of the thousand and one molecules. *Frontiers in Plant Science*, *7*(FEB2016), 1-17. https://doi.org/10.3389/fpls.2016.00019

Ardiansyah, Shirakawa, H., Koseki, T., Ohinata, K., Hashizume, K., & Komai, M. (2006). Erratum: Rice bran fractions improve blood pressure, lipid profile, and glucose metabolism in stroke-prone spontaneously hypertensive rats (Journal of Agricultural and Food Chemistry (2006) 54, (1914) DOI: 10.1021/jf068016h). *Journal of Agricultural and Food Chemistry*, *54*(24), 9277-9277. https://doi.org/10.1021/jf068016h

Asif, M. (2011). Health effects of omega-3,6,9 fatty acids: Perilla frutescens is a good example of plant oils. *Oriental Pharmacy & Experimental Medicine*, *11*(1), 51-59. https://doi.org/10.1007/s13596-011-0002-x

Attard, T. M., & Hunt, A. J. (2018). CHAPTER 3 Supercritical Carbon Dioxide Extraction of Lipophilic Molecules. In (pp. 40-76). The Royal Society of Chemistry. https://doi.org/10.1039/9781788013543-00040

Azlan, A., Prasad, K. N., Khoo, H. E., Abdul-Aziz, N., Mohamad, A., Ismail, A., & Amom, Z. (2010). Comparison of fatty acids, vitamin E and physicochemical properties of Canarium odontophyllum Miq. (dabai), olive and palm oils. *Journal of Food Composition and Analysis*, *23*(8), 772-776. https://doi.org/https://doi.org/10.1016/j.jfca.2010.03.026

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials : A review. *Journal of Food Engineering*, *117*(4), 426-436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Balbino, M. A., de Menezes, M. M., Eleoterio, I. C., Saczk, A. A., Okumura, L. L., Tristao, H. M., & de Oliveira, M. F. (2012). Voltammetric determination of Delta9-THC in glassy carbon electrode: An important contribution to forensic electroanalysis. *Forensic Sci Int*, *221*(1-3), 29-32. https://doi.org/10.1016/j.forsciint.2012.03.020

Baldino, L., Scognamiglio, M., & Reverchon, E. (2020). Supercritical fluid technologies applied to the extraction of compounds of industrial interest from Cannabis sativa L. and to their pharmaceutical formulations: A review. *Journal of*

Supercritical Fluids, *165*, 104960-104960. https://doi.org/10.1016/j.supflu.2020.104960

Baroni, A., Buommino, E., De Gregorio, V., Ruocco, E., Ruocco, V., & Wolf, R. (2012). Structure and function of the epidermis related to barrier properties. *Clinics in Dermatology*, *30*(3), 257-262. https://doi.org/10.1016/j.clindermatol.2011.08.007

Bartolomei, M., Bollati, C., Bellumori, M., Cecchi, L., Cruz-Chamorro, I., Santos-Sánchez, G., Ranaldi, G., Ferruzza, S., Sambuy, Y., Arnoldi, A., Mulinacci, N., & Lammi, C. (2021). Extra virgin olive oil phenolic extract on human hepatic HEPG2 and intestinal CACO-2 cells: Assessment of the antioxidant activity and intestinal trans-epithelial transport. *Antioxidants*, *10*(1), 1-20. https://doi.org/10.3390/antiox10010118

Benítez, R., Coronell, C., & Martin, J. (2018). Chemical Characterization Sacha Inchi (Plukenetia Volubilis) Seed: Oleaginosa Promising From the Colombian Amazon. *01*(01), 1-12.

Bergamaschi, M. M., Queiroz, R. H. C., Chagas, M. H. N., De Oliveira, D. C. G., De Martinis, B. S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A. E., Martín-Santos, R., Hallak, J. E. C., Zuardi, A. W., & Crippa, J. A. S. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-nave social phobia patients. *Neuropsychopharmacology*, *36*(6), 1219-1226. https://doi.org/10.1038/npp.2011.6

Bhargava, A., Shrivastava, P., & Tilwari, A. (2021). HPTLC analysis of Fumaria parviflora (Lam.) methanolic extract of whole plant. *Future Journal of Pharmaceutical Sciences*, *7*(1), 1-9. https://doi.org/10.1186/s43094-020-00150-x

Biniek, K., Levi, K., & Dauskardt, R. H. (2012). Solar UV radiation reduces the barrier function of human skin. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(42), 17111-17116. https://doi.org/10.1073/pnas.1206851109

Bonini, S. A., Premoli, M., Tambaro, S., Kumar, A., Maccarinelli, G., Memo, M., & Mastinu, A. (2018). Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *Journal of*

Ethnopharmacology, *227*(September), 300-315. https://doi.org/10.1016/j.jep.2018.09.004

Brenneisen, R. (2007). Chemistry and Analysis of Phytocannabinoids and Other Cannabis Constituents. In M. A. ElSohly (Ed.), (pp. 17-49). Humana Press. https://doi.org/10.1007/978-1-59259-947-9_2

Bridgeman, M. B., & Abazia, D. (2017). Medicinal Cannabis: History, Pharmacology, And Implications for the Acute Care Setting. *Pharmacy and Therapeutics*, *42*(3), 180-180.

Buckland, G., Mayén, A. L., Agudo, A., Travier, N., Navarro, C., Huerta, J. M., Chirlaque, M. D., Barricarte, A., Ardanaz, E., Moreno-Iribas, C., Marin, P., Quirós, J. R., Redondo, M. L., Amiano, P., Dorronsoro, M., Arriola, L., Molina, E., Sanchez, M. J., & Gonzalez, C. A. (2012). Olive oil intake and mortality within the Spanish population (EPIC-Spain). *American Journal of Clinical Nutrition*, *96*(1), 142-149. https://doi.org/10.3945/ajcn.111.024216

Bussmann, R. W., Batsatsashvili, K., Kikvidze, Z., Paniagua-Zambrana, N. Y., Khutsishvili, M., Maisaia, I., Sikharulidze, S., & Tchelidze, D. (2019). Cannabis sativa L.Cannabaceae. In K. Batsatsashvili, Z. Kikvidze, & R. Bussmann (Eds.), (pp. 1-4). Springer International Publishing. https://doi.org/10.1007/978-3-319-77088-8_33-2

Cantele, C., Bertolino, M., Bakro, F., Giordano, M., Jędryczka, M., & Cardenia, V. (2020). Antioxidant effects of hemp (Cannabis sativa L.) inflorescence extract in stripped linseed oil. *Antioxidants*, *9*(11), 1-18. https://doi.org/10.3390/antiox9111131

Capello, C., Fischer, U., & Hungerbühler, K. (2007). What is a green solvent? A comprehensive framework for the environmental assessment of solvents. *Green Chemistry*, *9*(9), 927-993. https://doi.org/10.1039/b617536h

Carter, G. T., Flanagan, A. M., Earleywine, M., Abrams, D. I., Aggarwal, S. K., & Grinspoon, L. (2011). Cannabis in palliative medicine: Improving care and reducing opioid-related morbidity. *American Journal of Hospice and Palliative Medicine*, *28*(5), 297-303. https://doi.org/10.1177/1049909111402318

Casiraghi, A., Roda, G., Casagni, E., Cristina, C., Musazzi, U. M., Franzè, S., Rocco, P., Giuliani, C., Fico, G., Minghetti, P., & Gambaro, V. (2018). Extraction Method and Analysis of Cannabinoids in Cannabis Olive Oil Preparations. *Planta Medica*, *84*(4), 242-249. https://doi.org/10.1055/s-0043-123074

Chagas, M. H. N., Zuardi, A. W., Tumas, V., Pena-Pereira, M. A., Sobreira, E. T., Bergamaschi, M. M., Dos Santos, A. C., Teixeira, A. L., Hallak, J. E. C., & Crippa, J. A. S. (2014). Effects of cannabidiol in the treatment of patients with Parkinson's disease: An exploratory double-blind trial. *Journal of Psychopharmacology*, *28*(11), 1088-1092. https://doi.org/10.1177/0269881114550355

Chakraborty, D., Das, J., Das, P., Bhattacharjee, S., & Das, S. (2017). Evaluation of the parameters affecting the extraction of sesame oil from sesame (Sesamum indicum L.) seed using soxhlet apparatus. *International Food Research Journal*, *24*, 691-695.

Chan, W. R., Magnus, K. E., & Watson, H. A. (1975). The Structure of Cannabitriol. *Experientia*, *32 (3)*(1972), 283-284.

Chandra, S., Lata, H., & ElSohly, M. A. (2017). Cannabis sativa L. - botany and biotechnology. *Cannabis sativa L. - Botany and Biotechnology*, 1-474. https://doi.org/10.1007/978-3-319-54564-6

Chemat, F., Abert Vian, M., Fabiano-Tixier, A. S., Nutrizio, M., Režek Jambrak, A., Munekata, P. E. S., Lorenzo, J. M., Barba, F. J., Binello, A., & Cravotto, G. (2020). A review of sustainable and intensified techniques for extraction of food and natural products. *Green Chemistry*, *22*(8), 2325-2353. https://doi.org/10.1039/c9gc03878g

Chen, B., McClements, D. J., & Decker, E. A. (2011). Minor components in food oils: A critical review of their roles on lipid oxidation chemistry in bulk oils and emulsions. *Critical Reviews in Food Science and Nutrition*, *51*(10), 901-916. https://doi.org/10.1080/10408398.2011.606379

Chen, H. M. M., & Meyers, S. P. (1982). Extraction of Astaxanthin Plgment from Crawfish Waste Using a Soy Oil Process. *Journal of Food Science*, *47*(3), 892-896. https://doi.org/10.1111/j.1365-2621.1982.tb12739.x

Chen, L., Hu, J. Y., & Wang, S. Q. (2012). The role of antioxidants in photoprotection: A critical review. *Journal of the American Academy of Dermatology*, *67*(5), 1013-1024. https://doi.org/10.1016/j.jaad.2012.02.009

Chiba, T. (1999). Prediction of Oxidative Stability Based on Various Chemical Properties for Refined Vegetable Oils. *Journal of Japan Oil Chemists' Society*, *48*(8), 781-786,815. https://doi.org/10.5650/jos1996.48.781

Chloe Fan, H. Y., Shaarani, S., Mamat, H., & Chiew, H. (2012). Frying stability of rice bran oil and palm olein. *International Food Research Journal*, *20*, 403-407.

Cisneros, F. H., Paredes, D., Arana, A., & Cisneros-zevallos, L. (2014). Chemical Composition, Oxidative Stability and Antioxidant Capacity of Oil Extracted from Roasted Seeds of Sacha-Inchi (Plukenetia volubilis L.). *Journal of Agricultural and Food Chemistry*, *62*, 5191-5197.

Citti, C., Battisti, U. M., Braghiroli, D., Ciccarella, G., Schmid, M., Vandelli, M. A., & Cannazza, G. (2018). A Metabolomic Approach Applied to a Liquid Chromatography Coupled to High-Resolution Tandem Mass Spectrometry Method (HPLC-ESI-HRMS/MS): Towards the Comprehensive Evaluation of the Chemical Composition of Cannabis Medicinal Extracts. *Phytochem Anal*, *29*(2), 144-155. https://doi.org/10.1002/pca.2722

Claussen, U., Von Spulak, F., & Korte, F. (1966). Chemical classification of plants. XXXI. Hashish. 10. Cannabichromene, a new hashish component. *Tetrahedron*, *22*(1964), 1477-1479.

Crippa, J. A. S., Nogueira Derenusson, G., Borduqui Ferrari, T., Wichert-Ana, L., Duran, F. L. S., Martin-Santos, R., Vinícius Simões, M., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z., Santos Filho, A., Freitas-Ferrari, M. C., McGuire, P. K., Zuardi, A. W., Busatto, G. F., & Hallak, J. E. C. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: A preliminary report. *Journal of Psychopharmacology*, *25*(1), 121-130. https://doi.org/10.1177/0269881110379283

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV radiation and the skin. *International Journal of Molecular Sciences*, *14*(6), 12222-12248. https://doi.org/10.3390/ijms140612222

Dachtler, M., van de Put, F., Stijn, F., Beindorff, C., & Fritsche, J. (2003). On-line LC-NMR-MS characterization of sesame oil extracts and assessment of their antioxidant activity. *European Journal of Lipid Science and Technology*, *105*, 488-496. https://doi.org/10.1002/ejlt.200300835

Damechki, M., Sotiropoulou, S., & Tsimidou, M. (2001). Antioxidant and pro-oxidant factors in oregano and rosemary gourmet olive oils. *Grasas y Aceites*, *52*(3-4), 207-213. https://doi.org/10.3989/gya.2001.v52.i3-4.359

Dawidowicz, A. L., Olszowy-Tomczyk, M., & Typek, R. (2021). CBG, CBD, Δ9-THC, CBN, CBGA, CBDA and Δ9-THCA as antioxidant agents and their intervention abilities in antioxidant action. *Fitoterapia*, *152*. https://doi.org/10.1016/j.fitote.2021.104915

de Melo, M. M. R., Silvestre, A. J. D., & Silva, C. M. (2014). Supercritical fluid extraction of vegetable matrices: Applications, trends and future perspectives of a convincing green technology. *The Journal of Supercritical Fluids*, *92*, 115-176. https://doi.org/https://doi.org/10.1016/j.supflu.2014.04.007

Deka, D., & Swami Hulle, N. R. (2021). Supercritical fluid extraction of Bhut Jolokia oleoresin and its quality analysis. *SN Applied Sciences*, *3*(2). https://doi.org/10.1007/s42452-021-04218-y

Deme, P., Narasimhulu, C. A., & Parthasarathy, S. (2018). Identification and evaluation of anti-inflammatory properties of aqueous components extracted from sesame (Sesamum indicum) oil. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1087-1088*(January), 61-69. https://doi.org/10.1016/j.jchromb.2018.04.029

Deng, L., Ng, L., Ozawa, T., & Stella, N. (2017). Quantitative analyses of synergistic responses between cannabidiol and DNA-damaging agents on the proliferation and viability of glioblastoma and neural progenitor cells in culture. *Journal of Pharmacology and Experimental Therapeutics*, *360*(1), 215-224. https://doi.org/10.1124/jpet.116.236968

Devarajan, S., Chatterjee, B., Urata, H., Zhang, B., Ali, A., Singh, R., & Ganapathy, S. (2016). A Blend of Sesame and Rice Bran Oils Lowers Hyperglycemia and

Improves the Lipids. *American Journal of Medicine*, *129*(7), 731-739. https://doi.org/10.1016/j.amjmed.2016.02.044

Devi, V., & Khanam, S. (2019). Study of ω-6 linoleic and ω-3 α-linolenic acids of hemp (Cannabis sativa) seed oil extracted by supercritical CO 2 extraction: CCD optimization. *Journal of Environmental Chemical Engineering*, *7*(1), 1-10. https://doi.org/10.1016/j.jece.2018.102818

Diamante, L. M., & Lan, T. (2014). Absolute Viscosities of Vegetable Oils at Different Temperatures and Shear Rate Range of 64.5 to 4835 s − 1. *Journal of Food Processing*, *2014*, 1-6. https://doi.org/10.1155/2014/234583

Dossa, K., Wei, X., Niang, M., Liu, P., Zhang, Y., & Wang, L. (2017). ScienceDirect Short communication Near-infrared reflectance spectroscopy reveals wide variation in major components of sesame seeds from Africa and Asia ☆. *The Crop Journal*, *6*(2), 202-206. https://doi.org/10.1016/j.cj.2017.10.003

Drinic, Z., Vidovic, S., Vladic, J., Koren, A., Kiprovski, B., & Sikora, V. (2018). Effect of extraction solvent on total polyphenols content and antioxidant activity of industrial hemp (Cannabis sativa L .). *Lekovite sirovine*, *38*, 17-21.

Edmondson, J. M., Armstrong, L. S., & Martinez, A. O. (1988). A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. *Journal of tissue culture methods*, *11*(1), 15-17. https://doi.org/10.1007/BF01404408

ElSohly, M. A., Radwan, M. M., Gul, W., Chandra, S., & Galal, A. (2017). Phytochemistry of Cannabis sativa L. In A. D. Kinghorn, H. Falk, S. Gibbons, & J. i. Kobayashi (Eds.), (pp. 1-36). Springer International Publishing. https://doi.org/10.1007/978-3-319-45541-9_1

ElSohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sciences*, *78*(5), 539-548. https://doi.org/10.1016/j.lfs.2005.09.011

Eyres, L. (2015). Eyres, Laurence. Frying oils: Selection, smoke points and potential deleterious effects for health [online]. Food New Zealand, Vol. 15, No. 1, Feb/Mar 2015: 30-31. Availability:

<http://search.informit.com.au.ezproxy.auckland.ac.nz/documentSummary;dn=131757870182463;res=IELNZC> ISSN: 1175-4621. [cited 09 Feb 17]. *Food New ZEaland*, *15(1)*, 30-31.

Fiorini, D., Scortichini, S., Bonacucina, G., Greco, N. G., Mazzara, E., Petrelli, R., Torresi, J., Maggi, F., & Cespi, M. (2020). Cannabidiol-enriched hemp essential oil obtained by an optimized microwave-assisted extraction using a central composite design. *Industrial Crops and Products*, *154*(March), 112688-112688. https://doi.org/10.1016/j.indcrop.2020.112688

Flachowsky, H., Schumann, E., Weber, W. E., & Peil, A. (2001). Application of AFLP for the detection of sex-specific markers in hemp. *Plant Breeding*, *120*(4), 305-309. https://doi.org/10.1046/j.1439-0523.2001.00620.x

Fowler, C. J., Gustafsson, S. B., Chung, S. C., Persson, E., Jacobsson, S. O. P., & Bergh, A. (2010). Targeting the endocannabinoid system for the treatment of cancer--a practical view. *Current topics in medicinal chemistry*, *10*(8), 814-827. https://doi.org/10.2174/156802610791164201

Friedman, D., & Sirven, J. I. (2017). Historical perspective on the medical use of cannabis for epilepsy: Ancient times to the 1980s. *Epilepsy and Behavior*, *70*, 298-301. https://doi.org/10.1016/j.yebeh.2016.11.033

Gallo-Molina, A. C., Castro-Vargas, H. I., Garzón-Méndez, W. F., Martínez Ramírez, J. A., Rivera Monroy, Z. J., King, J. W., & Parada-Alfonso, F. (2019). Extraction, isolation and purification of tetrahydrocannabinol from the Cannabis sativa L. plant using supercritical fluid extraction and solid phase extraction. *Journal of Supercritical Fluids*, *146*(January), 208-216. https://doi.org/10.1016/j.supflu.2019.01.020

Gaoni, Y., & Mechoulam, R. (1964). Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *Journal of the American Chemical Society*, *86*(8), 1646-1647. https://doi.org/10.1021/ja01062a046

García-Martínez, O., Mazzaglia, G., Sánchez-Ortiz, A., Ocaña-Peinado, F. M., & Rivas, A. (2014). Phenolic content of Sicilian virgin olive oils and their effect on MG-63 human osteoblastic cell proliferation. *Grasas y Aceites*, *65*(3). https://doi.org/10.3989/gya.0111141

Garmendia, F., Pando, R., & Ronceros, G. (2014). Efecto del aceite de sacha inchi (Plukenetia volúbilis L) sobre el perfil lipídico en pacientes con hiperlipoproteinemia. *Revista Peruana de Medicina Experimental y Salud Pública*, *28*(4), 5-8. https://doi.org/10.17843/rpmesp.2011.284.426

Gaston, O. A., Daniel, N. S., & Arnold, N. O. (2021). Physico-chemical properties of kernel from coconut (Cocos nucifera L.) varieties grown at the Kenyan Coast. *African Journal of Food Science*, *15*(8), 313-321. https://doi.org/10.5897/ajfs2021.2116

Ghani, N. A. A., Channip, A.-A., Hwa, P. C. H., Ja'afar, F., Yasin, H. M., & Usman, A. (2018). Physicochemical properties, antioxidant capacities, and metal contents of virgin coconut oil produced by wet and dry processes. *Food Science & Nutrition*, *6*(April), 1298-1306. https://doi.org/10.1002/fsn3.671

Gliszczyńska-Świgło, A., Sikorska, E., Khmelinskii, I., & Sikorski, M. (2007). TOCOPHEROL CONTENT IN EDIBLE PLANT OILS. *Polish Journal of Food and Nutrition Sciences*, *57*(4A), 157-161. http://journal.pan.olsztyn.pl/TOCOPHEROL-CONTENT-IN-EDIBLE-PLANT-OILS,98802,0,2.html

Gonzales, G. F., & Gonzales, C. (2014). A randomized, double-blind placebo-controlled study on acceptability, safety and efficacy of oral administration of sacha inchi oil (Plukenetia volubilis L.) in adult human subjects. *Food and Chemical Toxicology*, *65*, 168-176. https://doi.org/10.1016/j.fct.2013.12.039

Gonzalez-Aspajo, G., Belkhelfa, H., Haddioui-Hbabi, L., Bourdy, G., & Deharo, E. (2015). Sacha Inchi Oil (Plukenetia volubilis L.), effect on adherence of Staphylococus aureus to human skin explant and keratinocytes in vitro. *Journal of Ethnopharmacology*, *171*, 330-334. https://doi.org/10.1016/j.jep.2015.06.009

Good, P., Haywood, A., Gogna, G., Martin, J., Yates, P., Greer, R., & Hardy, J. (2019). Oral medicinal cannabinoids to relieve symptom burden in the palliative care of patients with advanced cancer: a double-blind, placebo controlled, randomised clinical trial of efficacy and safety of cannabidiol (CBD). *BMC Palliative Care*, *18*(1), 110. https://doi.org/10.1186/s12904-019-0494-6

Grijo, D. R., Bidoia, D. L., Nakamura, C. V., Osorio, I. V., & Cardozo-Filho, L. (2019). Analysis of the antitumor activity of bioactive compounds of Cannabis flowers extracted by green solvents. *The Journal of Supercritical Fluids*, *149*, 20-25.

Grijó, D. R., Vieitez Osorio, I. A., & Cardozo-Filho, L. (2018). Supercritical extraction strategies using CO2 and ethanol to obtain cannabinoid compounds from Cannabis hybrid flowers. *Journal of CO2 Utilization*, *28*(October), 174-180. https://doi.org/10.1016/j.jcou.2018.09.022

Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(14), 8268-8273. https://doi.org/10.1073/pnas.95.14.8268

Handayani, A. D., Sutrisno, Indraswati, N., & Ismadji, S. (2008). Extraction of astaxanthin from giant tiger (Panaeus monodon) shrimp waste using palm oil: studies of extraction kinetics and thermodynamic. *Bioresour Technol*, *99*(10), 4414-4419. https://doi.org/10.1016/j.biortech.2007.08.028

Hänel, K. H., Cornelissen, C., Lüscher, B., & Baron, J. M. (2013). Cytokines and the skin barrier. *International Journal of Molecular Sciences*, *14*(4), 6720-6745. https://doi.org/10.3390/ijms14046720

Hanus, L., & Krejci, Z. (1975). Isolation of two new cannabinoid acids from Cannabis sativa L. of Czechoslovak origin. *Acta Universitatis Palackianae Olomucensis Facultatis Medicae*, *No. 74*(January), 161-166.

Happyana, N., Agnolet, S., Muntendam, R., Van Dam, A., Schneider, B., & Kayser, O. (2013). Analysis of cannabinoids in laser-microdissected trichomes of medicinal Cannabis sativa using LCMS and cryogenic NMR. *Phytochemistry*, *87*, 51-59. https://doi.org/10.1016/j.phytochem.2012.11.001

Hartsel, J. A., Eades, J., Hickory, B., & Makriyannis, A. (2016). *Cannabis sativa and Hemp*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802147-7.00053-X

Hashimoto, M., Matsuzaki, K., Hossain, S., Ito, T., Wakatsuki, H., Tanabe, Y., Ohno, M., Kato, S., Yamashita, K., & Shido, O. (2021). Perilla seed oil enhances cognitive function and mental health in healthy elderly japanese individuals by

enhancing the biological antioxidant potential. *Foods*, *10*(5). https://doi.org/10.3390/foods10051130

Hazekamp, A., & Romano, L. L. (2013). Cannabis Oil: chemical evaluation of an upcoming cannabis-based medicine. *Cannabinoids*, *1*(1), 1-11.

Heiskanen, V., Pfiffner, M., & Partonen, T. (2020). Sunlight and health: shifting the focus from vitamin D3 to photobiomodulation by red and near-infrared light. *Ageing Research Reviews*, *61*(May). https://doi.org/10.1016/j.arr.2020.101089

Herrero, M., Mendiola, J. A., Cifuentes, A., & Ibáñez, E. (2010). Supercritical fluid extraction: Recent advances and applications. *Journal of Chromatography A*, *1217*(16), 2495-2511. https://doi.org/https://doi.org/10.1016/j.chroma.2009.12.019

Holick, M. F. (2016). Biological effects of sunlight, ultraviolet radiation, visible light, infrared radiation and Vitamin D for health. *Anticancer Research*, *36*(3), 1345-1356.

Hong, S., Sowndhararajan, K., Joo, T., Lim, C., Cho, H., Kim, S., Kim, G. Y., & Jhoo, J. W. (2015). Ethanol and supercritical fluid extracts of hemp seed (Cannabis sativa L.) increase gene expression of antioxidant enzymes in HepG2 cells. *Asian Pacific Journal of Reproduction*, *4*(2), 147-152. https://doi.org/10.1016/S2305-0500(15)30012-9

Huchelmann, A., Boutry, M., & Hachez, C. (2017). Plant glandular trichomes: Natural cell factories of high biotechnological interest. *Plant Physiology*, *175*(1), 6-22. https://doi.org/10.1104/pp.17.00727

Illam, S. P., Narayanankutty, A., Kandiyil, S. P., & Raghavamenon, A. C. (2021). Variations in natural polyphenols determine the anti-inflammatory potential of virgin coconut oils. *Journal of Food Science*, *86*(5), 1620-1628. https://doi.org/10.1111/1750-3841.15705

Iranloye, B., Oludare, G., & Olubiyi, M. (2013). Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. *Journal of Diabetes Mellitus*, *03*(04), 221-226. https://doi.org/10.4236/jdm.2013.34034

Islam, F., Gill, R. A., Ali, B., Farooq, M. A., Xu, L., Najeeb, U., & Zhou, W. (2016). Sesame. 135-147. https://doi.org/10.1016/B978-0-12-801309-0/00006-9

Janu, C., Kumar, D. R. S., Reshma, M. V., Jayamurthy, P., Sundaresan, A., & Nisha, P. (2014). Comparative study on the total phenolic content and radical scavenging activity of common edible vegetable oils. *Journal of Food Biochemistry*, *38*(1), 38-49. https://doi.org/10.1111/jfbc.12023

Japón-Luján, R., Janeiro, P., & De Castro, M. D. L. (2008). Solid-liquid transfer of biophenols from olive leaves for the enrichment of edible oils by a dynamic ultrasound-assisted approach. *Journal of Agricultural and Food Chemistry*, *56*(16), 7231-7235. https://doi.org/10.1021/jf800748p

Ji, W.-W., Li, R.-P., Li, M., Wang, S.-Y., Zhang, X., Niu, X.-X., Li, W., Yan, L., Wang, Y., Fu, Q., & Ma, S.-P. (2014). Antidepressant-like effect of essential oil of Perilla frutescens in a chronic, unpredictable, mild stress-induced depression model mice. *Chinese Journal of Natural Medicines*, *12*(10), 753-759. https://doi.org/https://doi.org/10.1016/S1875-5364(14)60115-1

Jimenez-lopez, C., Carpena, M., Lourenço-lopes, C., Gallardo-gomez, M., Lorenzo, J. M., Barba, F. J., Prieto, M. A., & Simal-gandara, J. (2020). Bioactive Compounds and Quality of Extra Virgin Olive Oil. *Foods*, *9*(8), 1014-1014.

Jurado-Ruiz, E., Álvarez-Amor, L., Varela, L. M., Berná, G., Parra-Camacho, M. S., Oliveras-Lopez, M. J., Martínez-Force, E., Rojas, A., Hmadcha, A., Soria, B., & Martín, F. (2019). Extra virgin olive oil diet intervention improves insulin resistance and islet performance in diet-induced diabetes in mice. *Scientific Reports*, *9*(1), 1-13. https://doi.org/10.1038/s41598-019-47904-z

Kang, C. D., & Sim, S. J. (2008). Direct extraction of astaxanthin from Haematococcus culture using vegetable oils. *Biotechnology Letters*, *30*(3), 441-444. https://doi.org/10.1007/s10529-007-9578-0

Kang, M.-h., Naito, M., Tsujihara, N., & Osawa, T. (1998). Sesamolin Inhibits Lipid Peroxidation in Rat Liver and Kidney. *The Journal of Nutrition*(February), 1018-1022.

Kangwan, N., Pratchayasakul, W., Kongkaew, A., Pintha, K., Chattipakorn, N., & Chattipakorn, C. S. (2021). Perilla Seed Oil Alleviates Gut Dysbiosis, Intestinal

Inflammation and Metabolic Disturbance in Obese-Insulin-Resistant Rats. *Nutrients*, *3141*(13), 1-14.

Kaplan, E. H., Offermann, E. A., Sievers, J. W., & Comi, A. M. (2017). Cannabidiol Treatment for Refractory Seizures in Sturge-Weber Syndrome. *Pediatric Neurology*, *71*, 18-23.e12. https://doi.org/10.1016/j.pediatrneurol.2017.02.009

Karas, J. A., Wong, L. J. M., Paulin, O. K. A., Mazeh, A. C., Hussein, M. H., Li, J., & Velkov, T. (2020). The antimicrobial activity of cannabinoids. *Antibiotics*, *9*(7), 1-10. https://doi.org/10.3390/antibiotics9070406

Karygianni, L., Cecere, M., Argyropoulou, A., Hellwig, E., Skaltsounis, A. L., Wittmer, A., Tchorz, J. P., & Al-Ahmad, A. (2019). Compounds from Olea europaea and Pistacia lentiscus inhibit oral microbial growth. *BMC Complementary and Alternative Medicine*, *19*(1), 1-10. https://doi.org/10.1186/s12906-019-2461-4

Keawkim, K., Lorjaroenphon, Y., Vangnai, K., & Jom, K. N. (2021). Metabolite–Flavor Profile, Phenolic Content, and Antioxidant Activity Changes in Sacha Inchi (Plukenetia volubilis L.) Seeds during Germination. *Foods*, *2476*(10), 1-15.

Khatoon, S., & Gopalakrishna, A. G. (2004). Fat-soluble nutraceuticals and fatty acid composition of selected Indian rice varieties. *Journal of the American Oil Chemists' Society*, *81*(10), 939-943. https://doi.org/10.1007/s11746-004-1005-5

King, J. W. (2014). Modern Supercritical Fluid Technology for Food Applications. *Annual Review of Food Science and Technology*, *5*(1), 215-238. https://doi.org/10.1146/annurev-food-030713-092447

Kinghorn, A. D., Falk, H., & Gibbons, S. (2017). *Phytocannabinoids* (Vol. 19). Springer International Publishing. https://doi.org/10.1007/978-3-319-45541-9

Kittipongpittaya, K., Panya, A., Cui, L., McClements, D. J., & Decker, E. A. (2014). Association Colloids Formed by Multiple Surface Active Minor Components and Their Effect on Lipid Oxidation in Bulk Oil. *Journal of the American Oil Chemists' Society*, *91*(11), 1955-1965. https://doi.org/10.1007/s11746-014-2541-z

Kizhiyedhatu Polachira, S., Jayalekshmy, A., & Chami, A. (2004). Free Radical Scavenging Behavior of Antioxidant Compounds of Sesame (Sesamum

indicum L .) in DPPH • System. *Journal of Agricultural and Food Chemistry*, 912-915.

Kodahl, N., & Sørensen, M. (2021). Sacha Inchi (Plukenetia volubilis L.) Is an Underutilized Crop with a Great Potential. *Agronomy*, *11*(6). https://doi.org/10.3390/agronomy11061066

Kornpointner, C., Sainz Martinez, A., Marinovic, S., Haselmair-Gosch, C., Jamnik, P., Schröder, K., Löfke, C., & Halbwirth, H. (2021). Chemical composition and antioxidant potential of Cannabis sativa L. roots. *Industrial Crops and Products*, *165*(March). https://doi.org/10.1016/j.indcrop.2021.113422

Korte, F., & Sieper, H. (1964). [ON THE CHEMICAL CLASSIFICATION OF PLANTS. XXIV. INVESTIGATION OF HASHISH CONSTITUENTS BY THIN-LAYER CHROMATOGRAPHY]. *Journal of chromatography*, *13*, 90-98. https://doi.org/10.1016/s0021-9673(01)95077-0

Kosgodage, U. S., Matewele, P., Awamaria, B., Kraev, I., Warde, P., Mastroianni, G., Nunn, A. V., Guy, G. W., Bell, J. D., Inal, J. M., & Lange, S. (2019). Cannabidiol Is a Novel Modulator of Bacterial Membrane Vesicles. *Frontiers in Cellular and Infection Microbiology*, *9*(September), 1-13. https://doi.org/10.3389/fcimb.2019.00324

Kouka, P., Priftis, A., Stagos, D., Angelis, A., Stathopoulos, P., Xinos, N., Skaltsounis, A. L., Mamoulakis, C., Tsatsakis, A. M., Spandidos, D. A., & Kouretas, D. (2017). Assessment of the antioxidant activity of an olive oil total polyphenolic fraction and hydroxytyrosol from a Greek Olea europea variety in endothelial cells and myoblasts. *International Journal of Molecular Medicine*, *40*(3), 703-712. https://doi.org/10.3892/ijmm.2017.3078

Kriese, U., Schumann, E., Weber, W. E., Beyer, M., Brühl, L., & Matthäus, B. (2004). Oil content, tocopherol composition and fatty acid patterns of the seeds of 51 Cannabis sativa L. genotypes. *Euphytica*, *137*(3), 339-351. https://doi.org/10.1023/B:EUPH.0000040473.23941.76

Krill, C., Rochfort, S., & Spangenberg, G. (2020). A High-Throughput Method for the Comprehensive Analysis of Terpenes and Terpenoids in Medicinal Cannabis Biomass. *Metabolites*, *10*(7). https://doi.org/10.3390/metabo10070276

Kuo, Y. H., Chiang, H. L., Wu, P. Y., Chu, Y., Chang, Q. X., Wen, K. C., Lin, C. Y., & Chiang, H. M. (2020). Protection against ultraviolet a-induced skin apoptosis and carcinogenesis through the oxidative stress reduction effects of n-(4-bromophenethyl) caffeamide, a propolis derivative. *Antioxidants*, *9*(4). https://doi.org/10.3390/antiox9040335

Lai, O. M., Jacoby, J. J., Leong, W. F., & Lai, W. T. (2019). *Nutritional studies of rice bran oil*. https://doi.org/10.1016/B978-0-12-812828-2.00002-0

Laznik, Ž., Košir, I. J., Košmelj, K., Murovec, J., Jagodič, A., Trdan, S., Ačko, D. K., & Flajšman, M. (2020). Effect of Cannabis sativa L. root, leaf and inflorescence ethanol extracts on the chemotrophic response of entomopathogenic nematodes. *Plant and Soil*, *455*(1-2), 367-379. https://doi.org/10.1007/s11104-020-04693-z

Lee, J., Lee, Y., & Choe, E. (2008). Effects of sesamol , sesamin , and sesamolin extracted from roasted sesame oil on the thermal oxidation of methyl linoleate. *LWT - Food Science and Technology*, *41*. https://doi.org/10.1016/j.lwt.2007.11.019

Lee, K. Y., Rahman, M. S., Kim, A. N., Jeong, E. J., Kim, B. G., Lee, M. H., Kim, H. J., & Choi, S. G. (2021). Effect of superheated steam treatment on yield, physicochemical properties and volatile profiles of perilla seed oil. *Lwt*, *135*(September 2020), 110240-110240. https://doi.org/10.1016/j.lwt.2020.110240

Li, N., Zhang, Z.-J., Li, X.-J., Li, H.-Z., Cui, L.-X., & He, D.-L. (2018). Microcapsules biologically prepared using Perilla frutescens (L.) Britt. essential oil and their use for extension of fruit shelf life [https://doi.org/10.1002/jsfa.8552]. *Journal of the Science of Food and Agriculture*, *98*(3), 1033-1041. https://doi.org/https://doi.org/10.1002/jsfa.8552

Li, W., Zhou, J., & Xu, Y. (2015). Study of the in vitro cytotoxicity testing of medical devices. *Biomedical reports*, *3*(5), 617-620. https://doi.org/10.3892/br.2015.481

Li, Y., Fabiano-Tixier, A. S., Ginies, C., & Chemat, F. (2014). Direct green extraction of volatile aroma compounds using vegetable oils as solvents: Theoretical and

experimental solubility study. *LWT - Food Science and Technology*, *59*(2P1), 724-731. https://doi.org/10.1016/j.lwt.2014.05.064

Llauradó Maury, G., Méndez Rodríguez, D., Hendrix, S., Escalona Arranz, J. C., Fung Boix, Y., Pacheco, A. O., García Díaz, J., Morris Quevedo, H. J., Ferrer Dubois, A., Isaac Aleman, E., Beenaerts, N., Méndez Santos, I. E., Orberá Ratón, T., Cos, P., & Cuypers, A. (2020). Antioxidants in plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in cuba. *Antioxidants*, *9*(11), 1-39. https://doi.org/10.3390/antiox9111048

Lokesha, S. B. (2021). Hepatoprotective Role of Virgin Coconut Oil Sanjana Basavanahally Lokesha and et al. *Journal of International Dental and Medical Research*.

Lopez, M. A. A., Solas, G. B., & Yu, A. I. (2021). The antiviral properties of a miracle oil (virgin coconut oil) from Cocos nucifera L.: a review. *5*(3), 5-19.

Ludwig, K., Rihko-Struckmann, L., Brinitzer, G., Unkelbach, G., & Sundmacher, K. (2021). β-Carotene extraction from Dunaliella salina by supercritical CO2. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-021-02399-y

Mai, H. C., Nguyen, D. C., Nhan, N. P. T., & Bach, L. G. (2020). Physico-chemical properties of sacha inchi (Plukenetia volubilis L.) seed oil from Vietnam. *Asian Journal of Chemistry*, *32*(2), 335-338.

Majdalawieh, A. F., & Mansour, Z. R. (2019). Sesamol, a major lignan in sesame seeds (Sesamum indicum): Anti-cancer properties and mechanisms of action. *European Journal of Pharmacology*, *855*(April), 75-89. https://doi.org/10.1016/j.ejphar.2019.05.008

Mandolino, G., Carboni, A., Forapani, S., Faeti, V., & Ranalli, P. (1999). Identification of DNA markers linked to the male sex in dioecious hemp (Cannabis sativa L.). *Theoretical and Applied Genetics*, *98*(1), 86-92. https://doi.org/10.1007/s001220051043

Maranduca, M. A., Branisteanu, D., Serban, D. N., Branisteanu, D. C., Stoleriu, G., Manolache, N., & Serban, I. L. (2019). Synthesis and physiological implications

of melanic pigments (review). *Oncology Letters*, *17*(5), 4183-4187. https://doi.org/10.3892/ol.2019.10071

Martinenghi, L. D., Jønsson, R., Lund, T., & Jenssen, H. (2020). Isolation, Purification, and Antimicrobial Characterization of Cannabidiolic Acid and Cannabidiol from Cannabis sativa L. *Biomolecules*, *10*(6). https://doi.org/10.3390/biom10060900

Matešić, N., Jurina, T., Benković, M., Panić, M., Valinger, D., Gajdoš Kljusurić, J., & Jurinjak Tušek, A. (2020). Microwave-assisted extraction of phenolic compounds from Cannabis sativa L.: optimization and kinetics study. *Separation Science and Technology (Philadelphia)*, *00*(00), 1-14. https://doi.org/10.1080/01496395.2020.1804938

Maurikaa, C. S., Jaganivash, B., & Shanmugasundaram, S. (2020). Comparative studies on physicochemical properties of virgin coconut oil (VCO) with different coconut oils. *International Journal of Chemical Studies*, *8*, 2433-2438. https://doi.org/10.22271/chemi.2020.v8.i6ai.11135

Mekky, R. H., Abdel-Sattar, E., Segura-Carretero, A., & Contreras, M. D. (2019). Phenolic Compounds from Sesame Cake and Antioxidant Activity: A New Insight for Agri-Food Residues' Significance for Sustainable Development. *Foods*, *8*(10). https://doi.org/10.3390/foods8100432

Moazzami, A., & Kamal-Eldin, A. (2009). *Sesame Seed Oil*. AOCS Press. https://doi.org/10.1016/B978-1-893997-97-4.50014-0

Moccia, S., Siano, F., Russo, G. L., Volpe, M. G., La Cara, F., Pacifico, S., Piccolella, S., & Picariello, G. (2020). Antiproliferative and antioxidant effect of polar hemp extracts (Cannabis sativa L., Fedora cv.) in human colorectal cell lines. *International Journal of Food Sciences and Nutrition*, *71*(4), 410-423. https://doi.org/10.1080/09637486.2019.1666804

Mohammed, N. K., Samir, Z. T., Jassim, M. A., & Saeed, S. K. (2021). Effect of different extraction methods on physicochemical properties, antioxidant activity, of virgin coconut oil. *Materials Today: Proceedings*, *42*, 2000-2005. https://doi.org/10.1016/j.matpr.2020.12.248

Monika, Kour, N., & Kaur, M. (2014). Antimicrobial Analysis of Leaves of Cannabis Sativa. *Journal of Science*, *4*(2), 123-127. www.journalofscience.net

Moreno, T., Montanes, F., Tallon, S. J., Fenton, T., & King, J. W. (2020). Extraction of cannabinoids from hemp (Cannabis sativa L.) using high pressure solvents: An overview of different processing options. *Journal of Supercritical Fluids*, *161*, 104850-104850. https://doi.org/10.1016/j.supflu.2020.104850

Mozaffarian, D., & Clarke, R. (2009). Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *European Journal of Clinical Nutrition*, *63*(2), S22-S33. https://doi.org/10.1038/sj.ejcn.1602976

Musetti, B., González-Ramos, H., González, M., Bahnson, E. M., Varela, J., & Thomson, L. (2020). Cannabis sativa extracts protect LDL from Cu2+-mediated oxidation. *Journal of Cannabis Research*, *2*(1). https://doi.org/10.1186/s42238-020-00042-0

Naftali, T., Bar-Lev Schleider, L., Dotan, I., Lansky, E. P., Sklerovsky Benjaminov, F., & Konikoff, F. M. (2013). Cannabis induces a clinical response in patients with crohn's disease: A prospective placebo-controlled study. *Clinical Gastroenterology and Hepatology*, *11*(10), 1276-1280.e1271. https://doi.org/10.1016/j.cgh.2013.04.034

Narayanan, D. L., Saladi, R. N., & Fox, J. L. (2010). Ultraviolet radiation and skin cancer. *International Journal of Dermatology*, *49*(9), 978-986. https://doi.org/10.1111/j.1365-4632.2010.04474.x

Nazzaro, F., Fratianni, F., Cozzolino, R., Martignetti, A., Malorni, L., Feo, V. D., Cruz, A. G., & D'acierno, A. (2019). Antibacterial activity of three extra virgin olive oils of the campania region, southern italy, related to their polyphenol content and composition. *Microorganisms*, *7*(9), 1-10. https://doi.org/10.3390/microorganisms7090321

Neubauer, D., Perković Benedik, M., & Osredkar, D. (2018). Cannabidiol for treatment of refractory childhood epilepsies: Experience from a single tertiary epilepsy center in Slovenia. *Epilepsy & Behavior*, *81*, 79-85. https://doi.org/https://doi.org/10.1016/j.yebeh.2018.02.009

Nguyen, L. C., Yang, D., Nicolaescu, V., Best, T. J., Ohtsuki, T., Chen, S.-N., Friesen, J. B., Drayman, N., Mohamed, A., Dann, C., Silva, D., Gula, H., Jones, K. A., Millis, J.

M., Dickinson, B. C., Tay, S., Oakes, S. A., Pauli, G. F., Meltzer, D. O., Randall, G., & Rosner, M. R. (2021). Cannabidiol Inhibits SARS-CoV-2 Replication and Promotes the Host Innate Immune Response. *bioRxiv : the preprint server for biology*, 1-15. https://doi.org/10.1101/2021.03.10.432967

Nierat, T., Musameh, S., & Abdel-Raziq, I. (2014). Temperature-dependence of olive oil viscosity. *Materials Science: An Indian Journal*, *11*(7), 233-238. https://www.researchgate.net/publication/263012034_Temperature-dependence_of_olive_oil_viscosity

Nissim, R., & Compton, R. G. (2015). Absorptive stripping voltammetry for cannabis detection. *Chem Cent J*, *9*, 41. https://doi.org/10.1186/s13065-015-0117-0

Novak, I., Mlakar, M., & Komorsky-Lovrić, Š. (2013). Voltammetry of Immobilized Particles of Cannabinoids. *Electroanalysis*, *25*(12), 2631-2636. https://doi.org/10.1002/elan.201300410

Nuapia, Y., Maraba, K., Tutu, H., Chimuka, L., & Cukrowska, E. (2021). In Situ Decarboxylation-Pressurized Hot Water Extraction for Selective Extraction of Cannabinoids from Cannabis sativa. Chemometric Approach. *Molecules*, *26*(11). https://doi.org/10.3390/molecules26113343

Olson, R. (2010). *Technology and Science in Ancient Civilizations*. ABC-CLIO. https://books.google.co.th/books?hl=id&lr=&id=0vP50ra6SYAC&oi=fnd&pg=PP1&dq=Olson+technology+and+science+in+ancient+2010&ots=KyOLYTro1R&sig=yILPEs9N5oq2BX5k4VG3o5ejOqM&redir_esc=y#v=onepage&q=Olson technology and science in ancient 2010&f=false

Owen, R. W., Haubner, R., Würtele, G., Hull, W. E., Spiegelhalder, B., & Bartsch, H. (2004). Olives and olive oil in cancer prevention. *European Journal of Cancer Prevention*, *13*(4), 319-326. https://doi.org/10.1097/01.cej.0000130221.19480.7e

Paduano, A., Caporaso, N., Santini, A., & Sacchi, R. (2014). Microwave and Ultrasound-Assisted Extraction of Capsaicinoids From Chili Peppers (Capsicum annuum L.) in Flavored Olive Oil. *Journal of Food Research*, *3*(4), 51-51. https://doi.org/10.5539/jfr.v3n4p51

Panich, U., Sittithumcharee, G., Rathviboon, N., & Jirawatnotai, S. (2016). Ultraviolet Radiation-Induced Skin Aging: The Role of DNA Damage and Oxidative Stress in Epidermal Stem Cell Damage Mediated Skin Aging. *Stem Cells Int*, *2016*, 7370642. https://doi.org/10.1155/2016/7370642

Park, H., Sa, K. J., Hyun, D. Y., Lee, S., & Lee, J. K. (2021). Identifying ssr markers related to seed fatty acid content in perilla crop (Perilla frutescens l.). *Plants*, *10*(7). https://doi.org/10.3390/plants10071404

Peeri, H., Shalev, N., Vinayaka, A. C., Nizar, R., Kazimirsky, G., Namdar, D., Anil, S. M., Belausov, E., Brodie, C., & Koltai, H. (2021). Specific compositions of cannabis sativa compounds have cytotoxic activity and inhibit motility and colony formation of human glioblastoma cells in vitro. *Cancers*, *13*(7). https://doi.org/10.3390/cancers13071720

Pertwee, R. G. (2012). Targeting the endocannabinoid system with cannabinoid receptor agonists: Pharmacological strategies and therapeutic possibilities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1607), 3353-3363. https://doi.org/10.1098/rstb.2011.0381

Phannasorn, W., Chariyakornkul, A., Sookwong, P., & Wongpoomchai, R. (2021). Comparative Studies on the Hepatoprotective Effect of White and Coloured Rice Bran Oil against Acetaminophen-Induced Oxidative Stress in Mice through Antioxidant- And Xenobiotic-Metabolizing Systems. *Oxidative Medicine and Cellular Longevity*, *2021*. https://doi.org/10.1155/2021/5510230

Pharmacopoeia, A. H. (2014). *Cannabis Inflorescence ; Cannabis spp.*

Pickering, E. E., Semple, S. J., Nazir, M. S., Murphy, K., Snow, T. M., Cummin, A. R., Moosavi, S. H., Guz, A., & Holdcroft, A. (2011). Cannabinoid effects on ventilation and breathlessness: A pilot study of efficacy and safety. *Chronic Respiratory Disease*, *8*(2), 109-118. https://doi.org/10.1177/1479972310391283

Pierre, F.-X., Souchon, I., Athes-Dutour, V., & Marin, M. (2002). Membrane-based solvent extraction of sulfur aroma compounds: influence of operating conditions on mass transfer coefficients in a hollow fiber contactor. *Desalination*, *148*(1-3), 199-204. https://www.sciencedirect.com/science/article/abs/pii/S0011916402006987#!

Pitts, M., Dorling, D., & Pattie, C. (2007). Oil for Food: the Global Story of Edible Lipids. *Journal of World-Systems Research*, *XIII*(1), 12-32. https://doi.org/10.5195/jwsr.2007.358

Poljšak, B., & Dahmane, R. (2012). Free radicals and extrinsic skin aging. *Dermatology Research and Practice*, *2012*. https://doi.org/10.1155/2012/135206

Poumay, Y., & Coquette, A. (2007). Modelling the human epidermis in vitro: tools for basic and applied research. *Archives of dermatological research*, *298*(8), 361-369. https://doi.org/10.1007/s00403-006-0709-6

Proksch, E., Brandner, J. M., & Jensen, J. M. (2008). The skin: An indispensable barrier. *Experimental Dermatology*, *17*(12), 1063-1072. https://doi.org/10.1111/j.1600-0625.2008.00786.x

Psaltopoulou, T., Kosti, R. I., Haidopoulos, D., Dimopoulos, M., & Panagiotakos, D. B. (2011). Olive oil intake is inversely related to cancer prevalence: A systematic review and a meta-analysis of 13800 patients and 23340 controls in 19 observational studies. *Lipids in Health and Disease*, *10*, 1-16. https://doi.org/10.1186/1476-511X-10-127

Pu, J., Bechtel, P. J., & Sathivel, S. (2010). Extraction of shrimp astaxanthin with flaxseed oil: Effects on lipid oxidation and astaxanthin degradation rates. *Biosystems Engineering*, *107*(4), 364-371. https://doi.org/10.1016/j.biosystemseng.2010.10.001

Purcaro, G., Barp, L., Beccaria, M., & Conte, L. S. (2016). Characterisation of minor components in vegetable oil by comprehensive gas chromatography with dual detection. *Food Chemistry*, *212*, 730-738. https://doi.org/10.1016/j.foodchem.2016.06.048

Qian, S., Guang-ming, C. A. I., Gui-xia, H. E., & Fang-lu, D. U. (2009). Study on the Chemical Constituents of Fruits of Cannabis Sativa L. *Natural Product Research & Development*, *21*(5).

Radoiu, M., Kaur, H., Bakowska-Barczak, A., & Splinter, S. (2020). Microwave-Assisted Industrial Scale Cannabis Extraction. *Technologies*, *8*(3), 45-45. https://doi.org/10.3390/technologies8030045

Raina, S., Thakur, A., Sharma, A., Pooja, D., & Minhas, A. P. (2020). Bactericidal activity of Cannabis sativa phytochemicals from leaf extract and their derived Carbon Dots and Ag@Carbon Dots. *Materials Letters*, *262*, 127122-127122. https://doi.org/10.1016/j.matlet.2019.127122

Raman, A., & London, K. C. (1998). Cultivation and Processing for Use. https://www.taylorfrancis.com/chapters/cannabis-plant-botany-cultivation-processing-use-amala-raman/e/10.1201/9780203304228-10

Reverchon, E., & De Marco, I. (2006). Supercritical fluid extraction and fractionation of natural matter. *The Journal of Supercritical Fluids*, *38*(2), 146-166. https://doi.org/https://doi.org/10.1016/j.supflu.2006.03.020

Ribeiro Grijó, D., Lazarin Bidoia, D., Vataru Nakamura, C., Vieitez Osorio, I., & Cardozo-Filho, L. (2019). Analysis of the antitumor activity of bioactive compounds of Cannabis flowers extracted by green solvents. *Journal of Supercritical Fluids*, *149*, 20-25. https://doi.org/10.1016/j.supflu.2019.03.012

Rincón-Cervera, M. Á., Valenzuela, R., Hernandez-Rodas, M. C., Barrera, C., Espinosa, A., Marambio, M., & Valenzuela, A. (2016). Vegetable oils rich in alpha linolenic acid increment hepatic n-3 LCPUFA, modulating the fatty acid metabolism and antioxidant response in rats. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *111*, 25-35. https://doi.org/10.1016/j.plefa.2016.02.002

Rochfort, S., Isbel, A., Ezernieks, V., Elkins, A., Vincent, D., Deseo, M. A., & Spangenberg, G. C. (2020). Utilisation of Design of Experiments Approach to Optimise Supercritical Fluid Extraction of Medicinal Cannabis. *Scientific Reports*, *10*(1), 1-7. https://doi.org/10.1038/s41598-020-66119-1

Rodriguez-Martin, N. M., Montserrat-De la Paz, S., Toscano, R., Grao-Cruces, E., Villanueva, A., Pedroche, J., Millan, F., & Millan-Linares, M. C. (2020). Hemp (Cannabis sativa l.) protein hydrolysates promote anti-inflammatory response in primary human monocytes. *Biomolecules*, *10*(5). https://doi.org/10.3390/biom10050803

Roheem, F. O., Ahmed, Q. U., Mat So'ad, S. Z., Shah, S. A. A., Latip, J., Alhassan, A. M., & Syed Mohammad, S. N. A. (2020). Assessment of Free radical scavenging and digestive enzyme inhibitory activities of extract, fractions and isolated

compounds from Tetracera macrophylla leaves. *Journal of Herbal Medicine*, *22*(March), 100351-100351. https://doi.org/10.1016/j.hermed.2020.100351

Rohman, A. (2014). Rice Bran Oil's Role in Health and Cooking. *Wheat and Rice in Disease Prevention and Health*, 481-490. https://doi.org/10.1016/B978-0-12-401716-0.00037-4

Rohman, A., Irnawati, Erwanto, Y., Lukitaningsih, E., Rafi, M., Fadzilah, N. A., Windarsih, A., Sulaiman, A., & Zakaria, Z. (2021). Virgin Coconut Oil: Extraction, Physicochemical Properties, Biological Activities and Its Authentication Analysis. *Food Reviews International*, *37*(1), 46-66. https://doi.org/10.1080/87559129.2019.1687515

Romero-Sandoval, E. A., Kolano, A. L., & Alvarado-Vázquez, P. A. (2017). Cannabis and Cannabinoids for Chronic Pain. *Current Rheumatology Reports*, *19*(11). https://doi.org/10.1007/s11926-017-0693-1

Romeu, M., Rubió, L., Sánchez-Martos, V., Castañer, O., De La Torre, R., Valls, R. M., Ras, R., Pedret, A., Catalán, Ú., López De Las Hazas, M. D. C., Motilva, M. J., Fitó, M., Solà, R., & Giralt, M. (2016). Virgin Olive Oil Enriched with Its Own Phenols or Complemented with Thyme Phenols Improves DNA Protection against Oxidation and Antioxidant Enzyme Activity in Hyperlipidemic Subjects. *Journal of Agricultural and Food Chemistry*, *64*(9), 1879-1888. https://doi.org/10.1021/acs.jafc.5b04915

Rosas-Quina, Y. E., & Mejía-Nova, F. C. (2021). Supercritical fluid extraction with cosolvent of alkaloids from Lupinus mutabilis Sweet and comparison with conventional method. *Journal of Food Process Engineering*(January), 1-10. https://doi.org/10.1111/jfpe.13657

Ross, S. A., ElSohly, M. A., Sultana, G. N. N., Mehmedic, Z., Hossain, C. F., & Chandra, S. (2005). Flavonoid glycosides and cannabinoids from the pollen of Cannabis sativa L. *Phytochemical Analysis*, *16*(1), 45-48. https://doi.org/10.1002/pca.809

Rovetto, L. J., & Aieta, N. V. (2017). Supercritical carbon dioxide extraction of cannabinoids from Cannabis sativa L. *The Journal of Supercritical Fluids*, *129*, 16-27. https://doi.org/10.1016/j.supflu.2017.03.014

Rožanc, J., Kotnik, P., Milojević, M., Gradišnik, L., Hrnčič, M. K., Knez, Ž., & Maver, U. (2021). Different cannabis sativa extraction methods result in different biological activities against a colon cancer cell line and healthy colon cells. *Plants*, *10*(3), 1-16. https://doi.org/10.3390/plants10030566

Russo, E. B. (2017). Cannabis and epilepsy: An ancient treatment returns to the fore. *Epilepsy and Behavior*, *70*, 292-297. https://doi.org/10.1016/j.yebeh.2016.09.040

Saewan, N., & Jimtaisong, A. (2015). Natural products as photoprotection. *Journal of Cosmetic Dermatology*, *14*(1), 47-63. https://doi.org/10.1111/jocd.12123

Saikia, D., & Deka, S. (2011). Cereals: From staple food to nutraceuticals. *International Food Research Journal*, *18*, 21-30.

Sakamoto, K., Abe, T., Matsuyama, T., Yoshida, S., Ohmido, N., Fukui, K., & Satoh, S. (2005). RAPD markers encoding retrotransposable elements are linked to the male sex in Cannabis sativa L. *Genome*, *48*(5), 931-936. https://doi.org/10.1139/g05-056

Sakamoto, K., Shimomura, K., Komeda, Y., Kamada, H., & Satoh, S. (1995). A Male-Associated DNA Sequence in a Dioecious Plant, Cannabis sativa L. *Plant and Cell Physiology*, *36*(8), 1549-1554. https://doi.org/10.1093/oxfordjournals.pcp.a078920

Salian, V., & Shetty, P. (2018). Coconut oil and virgin coconut oil: An insight into its oral and overall health benefits. *Journal of Clinical and Diagnostic Research*, *12*(1), ZE01-ZE03. https://doi.org/10.7860/JCDR/2018/31409.11051

Sarwar, A., Vunguturi, S., & Ferdose, A. (2016). A Study on Smoke Point and Peroxide Values of different

widely used Edible Oils. *International Journal of Engineering Technology Science and Research*, *3*(5), 271-273.

Seo, M.-D., Kang, T. J., Lee, C. H., Lee, A.-Y., & Noh, M. (2012). HaCaT Keratinocytes and Primary Epidermal Keratinocytes Have Different Transcriptional Profiles of Cornified Envelope-Associated Genes to T Helper Cell Cytokines.

Biomolecules & therapeutics, *20*(2), 171-176. https://doi.org/10.4062/biomolther.2012.20.2.171

Shakun, A. S., Vorobyova, V. I., Chygyrynets, O. E., & Skiba, M. I. (2020). Influence of Solvent on the Component Composition and Antioxidant Properties of Apricot Cake (Prunus armeniaca L.) Extracts. *Journal of Chemistry*, *2020*, 2913454. https://doi.org/10.1155/2020/2913454

Shani, A., & Mechoulam, R. (1974). Cannabielsoic acids. Isolation and synthesis by a novel oxidative cyclization. *Tetrahedron*, *30*(15), 2437-2446. https://doi.org/10.1016/S0040-4020(01)97114-5

Shinde, V., & Mahadi, K. (2019). Supercritical fluid extraction: A new technology to herbals. *Int. J. Herb. Med*, *7*, 27-34.

Siddiqui, N., & Ahmad, A. (2013). A Study on Viscosity, Surface Tension and Volume Flow Rate of Some Edible and Medicinal Oils. *International Journal of Science, Environment, and Technology*, *2*(6), 1318-1326.

Silenzi, A., Giovannini, C., Scazzocchio, B., Varì, R., D'Archivio, M., Santangelo, C., & Masella, R. (2020). Extra virgin olive oil polyphenols: biological properties and antioxidant activity. *Pathology*, 225-233. https://doi.org/10.1016/b978-0-12-815972-9.00022-6

Silva, B. S., & Schmiele, M. (2021). From olive to olive oil: a general approach. *Research, Society and Development*, *10*(3), e32210313408-e32210313408. https://doi.org/10.33448/rsd-v10i3.13408

Sisodiya, D., & Shrivastava, P. (2017). Qualitative and quantitative estimation of bioactive compounds of Euphorbia thymifolia L. *Asian Journal of Pharmaceutical Education and Research*, *6*(3), 34-43.

Smeriglio, A., Trombetta, D., Alloisio, S., Cornara, L., Denaro, M., Garbati, P., Grassi, G., & Circosta, C. (2020). Promising in vitro antioxidant, anti-acetylcholinesterase and neuroactive effects of essential oil from two non-psychotropic Cannabis sativa L. biotypes. *Phytotherapy Research*, *34*(9), 2287-2302. https://doi.org/10.1002/ptr.6678

Smith, J. P., Sutcliffe, O. B., & Banks, C. E. (2015). An overview of recent developments in the analytical detection of new psychoactive substances (NPSs). *Analyst*, *140*(15), 4932-4948. https://doi.org/10.1039/c5an00797f

Sobstyl, E., Szopa, A., Ekiert, H., Gnat, S., Typek, R., & Choma, I. M. (2020). Effect directed analysis and TLC screening of Schisandra chinensis fruits. *Journal of Chromatography A*, *1618*, 460942-460942. https://doi.org/10.1016/j.chroma.2020.460942

Someya, T., & Amagai, M. (2019). Toward a new generation of smart skins. *Nature Biotechnology*, *37*(4), 382-388. https://doi.org/10.1038/s41587-019-0079-1

Suja, K. P., Jayalekshmy, A., & Arumughan, C. (2005). In vitro studies on antioxidant activity of lignans isolated from sesame cake extract. *Journal of the Science of Food and Agriculture*, *1783*(June 2004), 1779-1783. https://doi.org/10.1002/jsfa.2170

Sun, M., & Temelli, F. (2006). Supercritical carbon dioxide extraction of carotenoids from carrot using canola oil as a continuous co-solvent. *Journal of Supercritical Fluids*, *37*(3), 397-408. https://doi.org/10.1016/j.supflu.2006.01.008

Suwanangul, S., Alashi, M. A., Aluko, R. E., Tochampa, W., & Ruttarattanamongkol, K. (2021). Inhibition of α-amylase, α-glucosidase and pancreatic lipase activities in vitro by sacha inchi (Plukenetia volubilis l.) protein hydrolysates and their fractionated peptides. *Maejo International Journal of Science and Technology*, *15*(1), 13-26.

Szaflarski, J. P., Bebin, E. M., Cutter, G., DeWolfe, J., Dure, L. S., Gaston, T. E., Kankirawatana, P., Liu, Y., Singh, R., Standaert, D. G., Thomas, A. E., & Ver Hoef, L. W. (2018). Cannabidiol improves frequency and severity of seizures and reduces adverse events in an open-label add-on prospective study. *Epilepsy and Behavior*, *87*, 131-136. https://doi.org/10.1016/j.yebeh.2018.07.020

Tallon, S., Moreno, T., Montanes, F., & Watson, M. (2018). Extraction and fractionation of cannabinoids from Cannabis Sativa. Chemeca Conference, New Zealand.

Tan, Z., Clomburg, J. M., & Gonzalez, R. (2018). Synthetic Pathway for the Production of Olivetolic Acid in Escherichia coli. *ACS Synthetic Biology*, *7*(8), 1886-1896. https://doi.org/10.1021/acssynbio.8b00075

Tiono, R. D., Santoso, S. P., Angkawijaya, A. E., Yuliana, M., Putro, J. N., Lie, J., Soetaredjo, F. E., Irawaty, W., & Ismadji, S. (2021). Investigation on supercritical CO2 extraction of black nightshade berries (Solanum nigrum linn.). *Biointerface Research in Applied Chemistry*, *11*(5), 13502-13515. https://doi.org/10.33263/BRIAC115.1350213515

Törjék, O., Bucherna, N., Kiss, E., Homoki, H., Finta-Korpelová, Z., Bócsa, I., Nagy, I., & Heszky, L. E. (2002). Novel male-specific molecular markers (MADC5, MADC6) in hemp. *Euphytica*, *127*(2), 209-218. https://doi.org/10.1023/A:1020204729122

Turner, C. E., Elsohly, M. A., & Boeren, E. G. (1980). Constituents of cannabis sativa l. xvii. a review of the natural constituents. *Journal of Natural Products*, *43*(2), 169-234. https://doi.org/10.1021/np50008a001

Veggi, P. C., Martinez, J., & Meireles, M. A. A. (2013). Fundamentals of Microwave Extraction. In F. Chemat & G. Cravotto (Eds.), (pp. 15-52). Springer US. https://doi.org/10.1007/978-1-4614-4830-3_2

Velasco, G., Sánchez, C., & Guzmán, M. (2012). Towards the use of cannabinoids as antitumour agents. *Nature Reviews Cancer*, *12*(6), 436-444. https://doi.org/10.1038/nrc3247

Veress, T., Szanto, J. I., & Leiztner, L. (1990). Determination of cannabinoid acids by high-performance liquid chromatography of their neutral derivatives formed bY thermal decarboxylation I . Study of the decarboxylation. *Journal of Chr*, *520*, 339-347.

Villarino, B. J., Dy, L. M., & Lizada, M. C. C. (2007). Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. *LWT - Food Science and Technology*, *40*(2), 193-199. https://doi.org/10.1016/j.lwt.2005.11.007

Wacal, C., Ogata, N., Basalirwa, D., Sasagawa, D., Kato, M., Handa, T., Masunaga, T., Yamamoto, S., & Nishihara, E. (2019). Fatty Acid Composition of Sesame (Sesamum indicum L.) Seeds in Relation to Yield and Soil Chemical Properties

on Continuously Monocropped Upland Fields Converted from Paddy Fields. *Agronomy*, *9*(12), 1-19. https://doi.org/10.3390/agronomy9120801

Wang, H., Guo, L., Liu, L., Han, B., & Niu, X. (2021). Composite chitosan films prepared using nisin and Perilla frutescense essential oil and their use to extend strawberry shelf life. *Food Bioscience*, *41*(December 2019), 101037-101037. https://doi.org/10.1016/j.fbio.2021.101037

Wang, M., Wang, Y. H., Avula, B., Radwan, M. M., Wanas, A. S., Van Antwerp, J., Parcher, J. F., Elsohly, M. A., & Khan, I. A. (2016). Decarboxylation Study of Acidic Cannabinoids: A Novel Approach Using Ultra-High-Performance Supercritical Fluid Chromatography/Photodiode Array-Mass Spectrometry. *Cannabis and Cannabinoid Research*, *1*(1), 262-271. https://doi.org/10.1089/can.2016.0020

Wang, X., Zhang, Z., Li, H., Hou, T., Zhao, Y., & Li, H. (2021). Effects of ethanol, activated carbon, and activated kaolin on perilla seed oil: Volatile organic compounds, physicochemical characteristics, and fatty acid composition [https://doi.org/10.1111/1750-3841.15907]. *Journal of Food Science*, *86*(10), 4393-4404. https://doi.org/https://doi.org/10.1111/1750-3841.15907

Ware, M. A., Wang, T., Shapiro, S., Robinson, A., Ducruet, T., Huynh, T., Gamsa, A., Bennett, G. J., & Collet, J. P. (2010). Smoked cannabis for chronic neuropathic pain: A randomized controlled trial. *Cmaj*, *182*(14). https://doi.org/10.1503/cmaj.091414

Warinhomhoun, S., Muangnoi, C., Buranasudja, V., Mekboonsonglarp, W., Rojsitthisak, P., Likhitwitayawuid, K., & Sritularak, B. (2021). Antioxidant Activities and Protective Effects of Dendropachol, a New Bisbibenzyl Compound from Dendrobium pachyglossum, on Hydrogen Peroxide-Induced Oxidative Stress in HaCaT Keratinocytes. *Antioxidants*, *10*(2). https://doi.org/10.3390/antiox10020252

Webster Barrie, G. R., & Sarna, L. P. (2002). Cannabinoid extraction method. *United States Patent*, *US 6,403,1*(June), 5-5. https://patents.google.com/patent/US6403126B1/en

Wong, B. S., Camilleri, M., Eckert, D., Carlson, P., Ryks, M., Burton, D., & Zinsmeister, A. R. (2012). Randomized pharmacodynamic and pharmacogenetic trial of dronabinol effects on colon transit in irritable bowel syndrome-diarrhea. *Neurogastroenterology and Motility*, *24*(4). https://doi.org/10.1111/j.1365-2982.2011.01874.x

Xiang, C., Xu, Z., Liu, J., Li, T., Yang, Z., & Ding, C. (2017). Quality, composition, and antioxidant activity of virgin olive oil from introduced varieties at Liangshan. *LWT - Food Science and Technology*, *78*, 226-234. https://doi.org/10.1016/j.lwt.2016.12.029

Xu, D., Hao, J., Wang, Z., Liang, D., Wang, J., Ma, Y., & Zhang, M. (2021). Physicochemical properties, fatty acid compositions, bioactive compounds, antioxidant activity and thermal behavior of rice bran oil obtained with aqueous enzymatic extraction. *LWT - Food Science and Technology*, *149*(11), 111817-111817. https://doi.org/10.1016/j.lwt.2021.111817

Yan, X., Tang, J., Dos Santos Passos, C., Nurisso, A., Simoes-Pires, C. A., Ji, M., Lou, H., & Fan, P. (2015). Characterization of Lignanamides from Hemp (Cannabis sativa L.) Seed and Their Antioxidant and Acetylcholinesterase Inhibitory Activities. *Journal of Agricultural and Food Chemistry*, *63*(49), 10611-10619. https://doi.org/10.1021/acs.jafc.5b05282

Yara-Varón, E., Li, Y., Balcells, M., Canela-Garayoa, R., Fabiano-Tixier, A. S., & Chemat, F. (2017). Vegetable oils as alternative solvents for green oleo-extraction, purification and formulation of food and natural products. *Molecules*, *22*(9), 1-24. https://doi.org/10.3390/molecules22091474

Yonbawi, A. R., Abdallah, H. M., Alkhilaiwi, F. A., Koshak, A. E., & Heard, C. M. (2021). Anti-Proliferative, Cytotoxic and Antioxidant Properties of the Methanolic Extracts of Five Saudi Arabian Flora with Folkloric Medicinal Use: Aizoon canariense, Citrullus colocynthis, Maerua crassifolia, Rhazya stricta and Tribulus macropterus. *Plants (Basel, Switzerland)*, *10*(10), 2073. https://doi.org/10.3390/plants10102073

Yoon, Y.-c., Kim, B.-h., Kim, J.-k., Lee, J.-h., Park, Y.-e., Kwon, G.-s., Hwang, H. S., & Lee, J.-b. (2018). Verification of Biological Activities and Tyrosinase Inhibition of

Ethanol Extracts from Hemp Seed (Cannabis sativa L.) Fermented with Lactic Acid Bacteria. *Journal of Life Science*, *28*(6), 688-696. https://doi.org/10.5352/JLS.2018.28.6.688

Yubero-Serrano, E. M., Lopez-Moreno, J., Gomez-Delgado, F., & Lopez-Miranda, J. (2019). Extra virgin olive oil: More than a healthy fat. *European Journal of Clinical Nutrition*, *72*, 8-17. https://doi.org/10.1038/s41430-018-0304-x

Zajicek, J. P., Hobart, J. C., Slade, A., Barnes, D., & Mattison, P. G. (2012). MUltiple sclerosis and extract of cannabis: Results of the MUSEC trial. *Journal of Neurology, Neurosurgery and Psychiatry*, *83*(11), 1125-1132. https://doi.org/10.1136/jnnp-2012-302468

Zhang, H. X., Tian, Y. H., Guan, J., Xie, Q. M., & Zhao, Y. Q. (2021). The anti-tussive, anti-inflammatory effects and sub-chronic toxicological evaluation of perilla seed oil. *Journal of the Science of Food and Agriculture*, *101*(4), 1419-1427. https://doi.org/10.1002/jsfa.10755

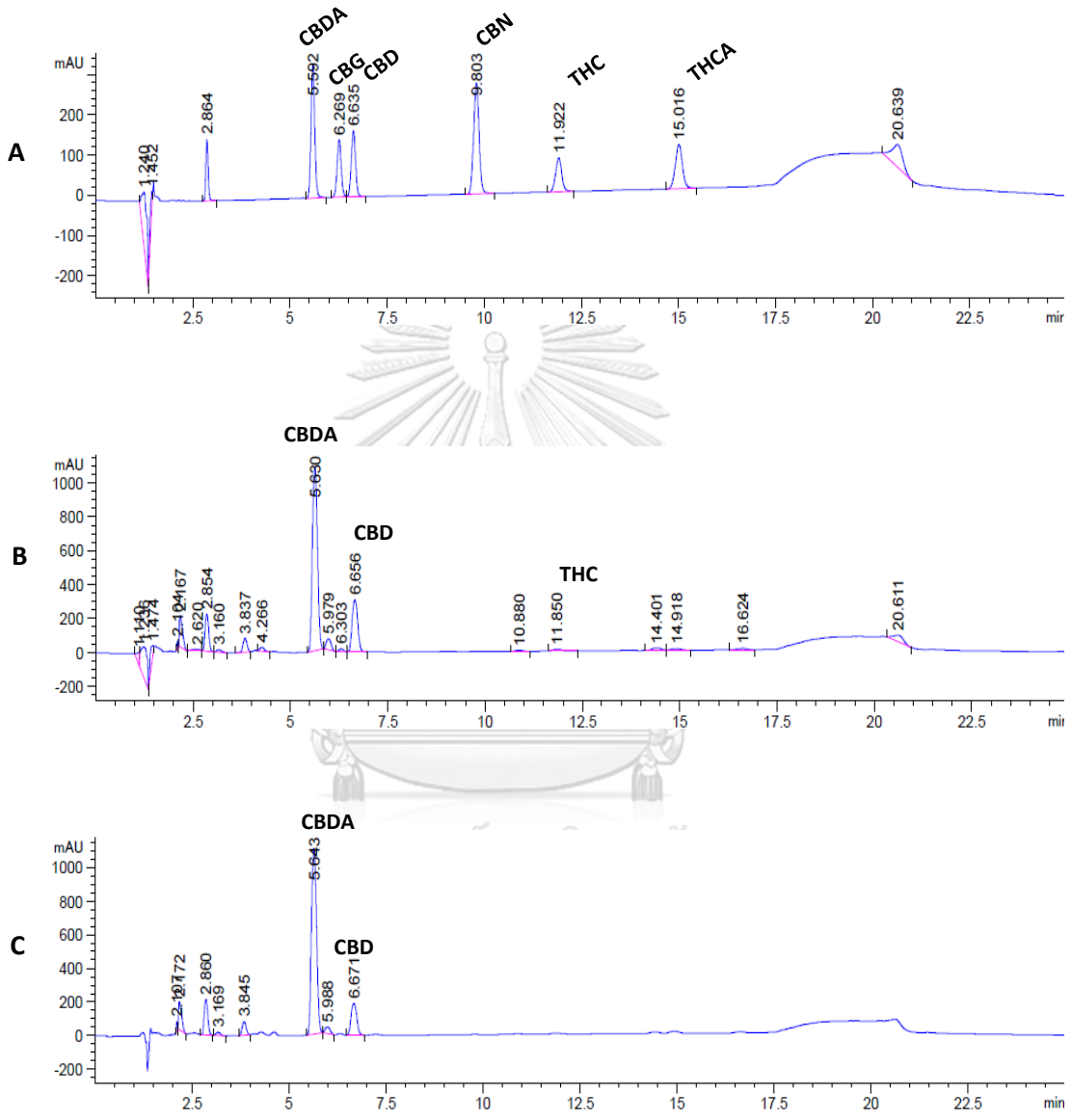
Zuardi, A. W. (2006). History of cannabis as a medicine: A review. *Revista Brasileira de Psiquiatria*, *28*(2), 153-157. https://doi.org/10.1590/S1516-44462006000200015

APPENDIX

Physicochemical properties of vegetable oils

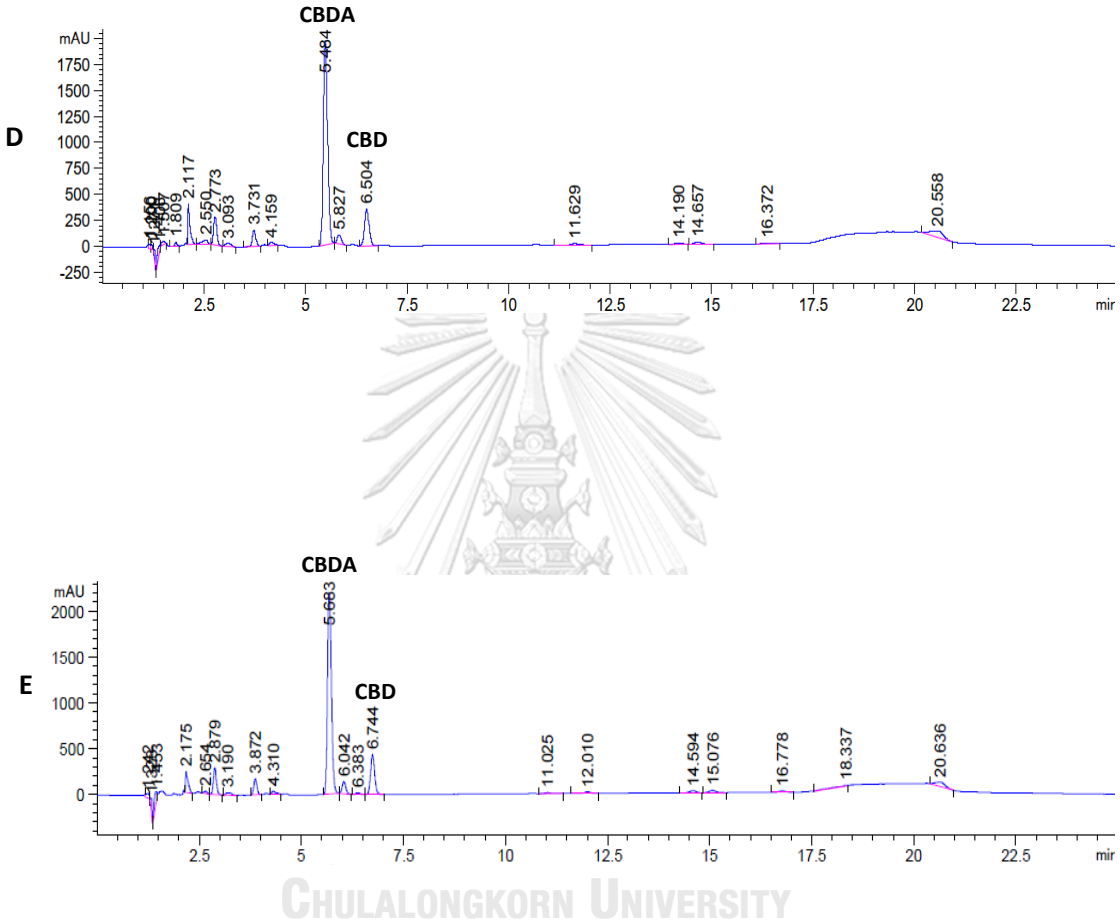
Vegetable Oil	Peroxide Value (meq O_2/kg)	Saponification Value (mg KOH/g)	Iodine Value (g I_2/100 g)	Acid Value (mg KOH/g)	Viscosity (cP)	Smoke Point (°C)	
Virgin Coconut Oil	2.2	259	0.97	2.7	50.7	170	(Eyres, 2015; Ghani et al., 2018; Maurikaa et al., 2020)
Olive Oil	7.98	189.30	83.1	0.84	60.7	190	(Azlan et al., 2010; Nierat et al., 2014; Sarwar et al., 2016)
Perilla Seed Oil	1.86	200.14	180.27	0.19	89	250	(Lee et al., 2021; X. Wang et al., 2021)
Sacha Inchi Oil	1.78	183.5	192.4	2.05	88	225	(Mai et al., 2020)
Sesame Seed Oil	4.8	190	112	0.78	36.5	232	(Chakraborty et al., 2017; Siddiqui & Ahmad, 2013)
Rice Bran Oil	8.15	184.87	106.31	7.97	39.80	235	(Chloe Fan et al., 2012; Diamante & Lan, 2014; Xu et al., 2021)

HPLC chromatogram of *C. sativa* L. strain CBD Charlotte's Angel female flower organic Solvent extracts (non-decarboxylated sample)



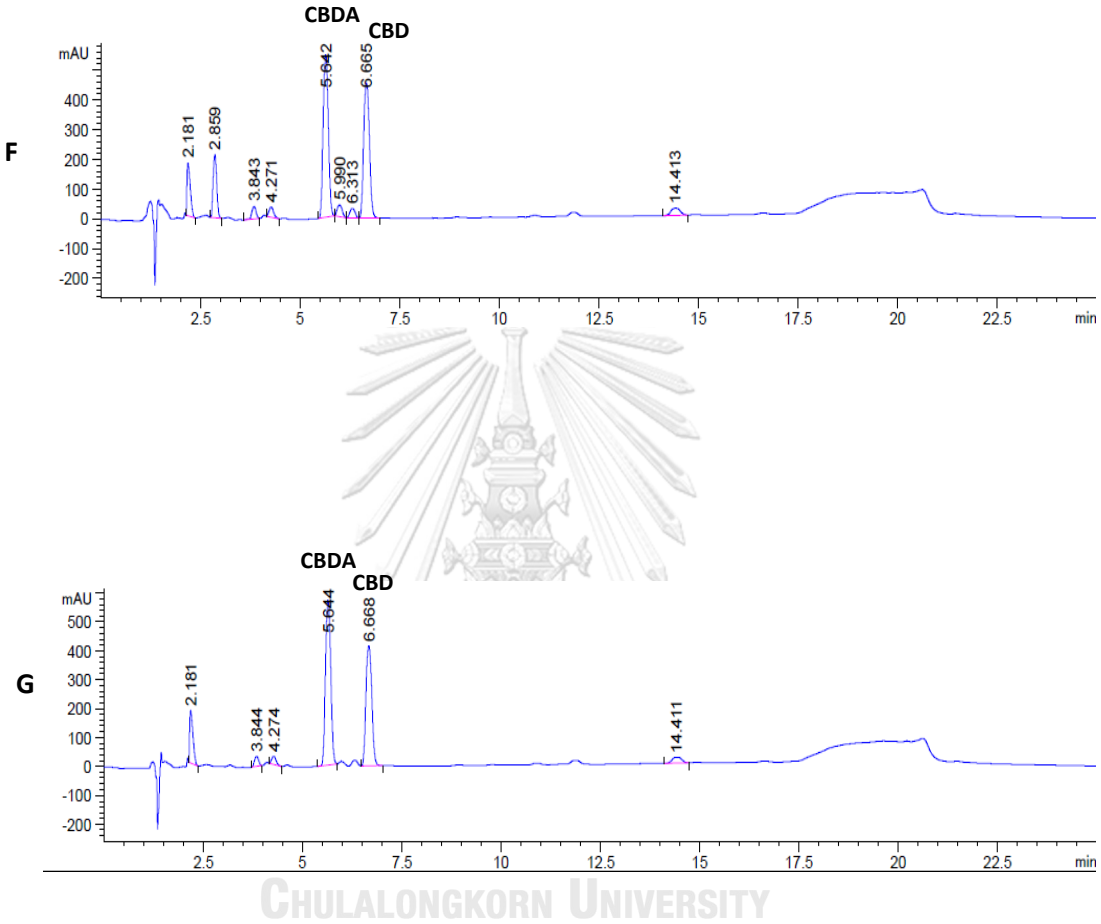
(A) cannabinoids standard; (B) ethanol extract; (C) hexane extract

HPLC chromatogram of *C. sativa* L. strain CBD Charlotte's Angel female flower vegetable oil extracts (non-decarboxylated sample)



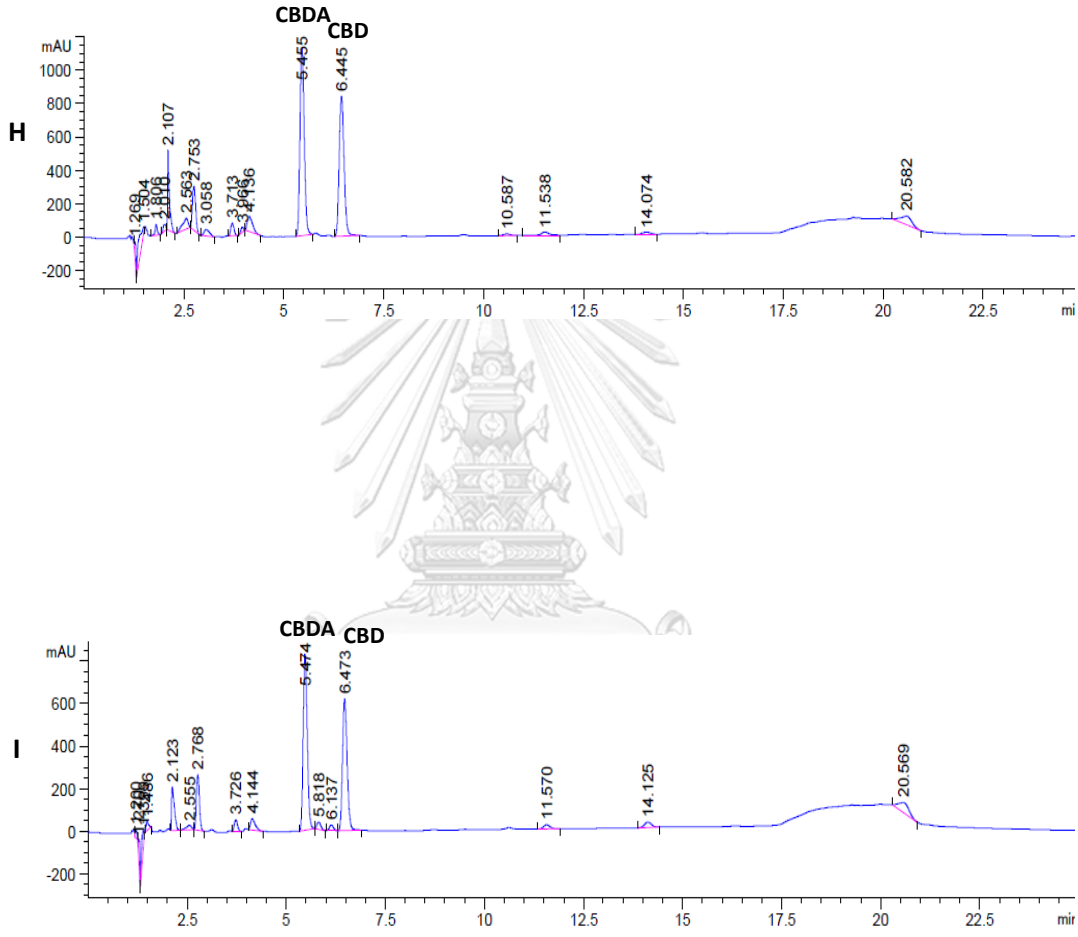
(D) virgin coconut oil; (E) olive oil

HPLC chromatogram of *C. sativa* L. strain CBD Charlotte's Angel female flower organic solvent extracts (decarboxylated sample)



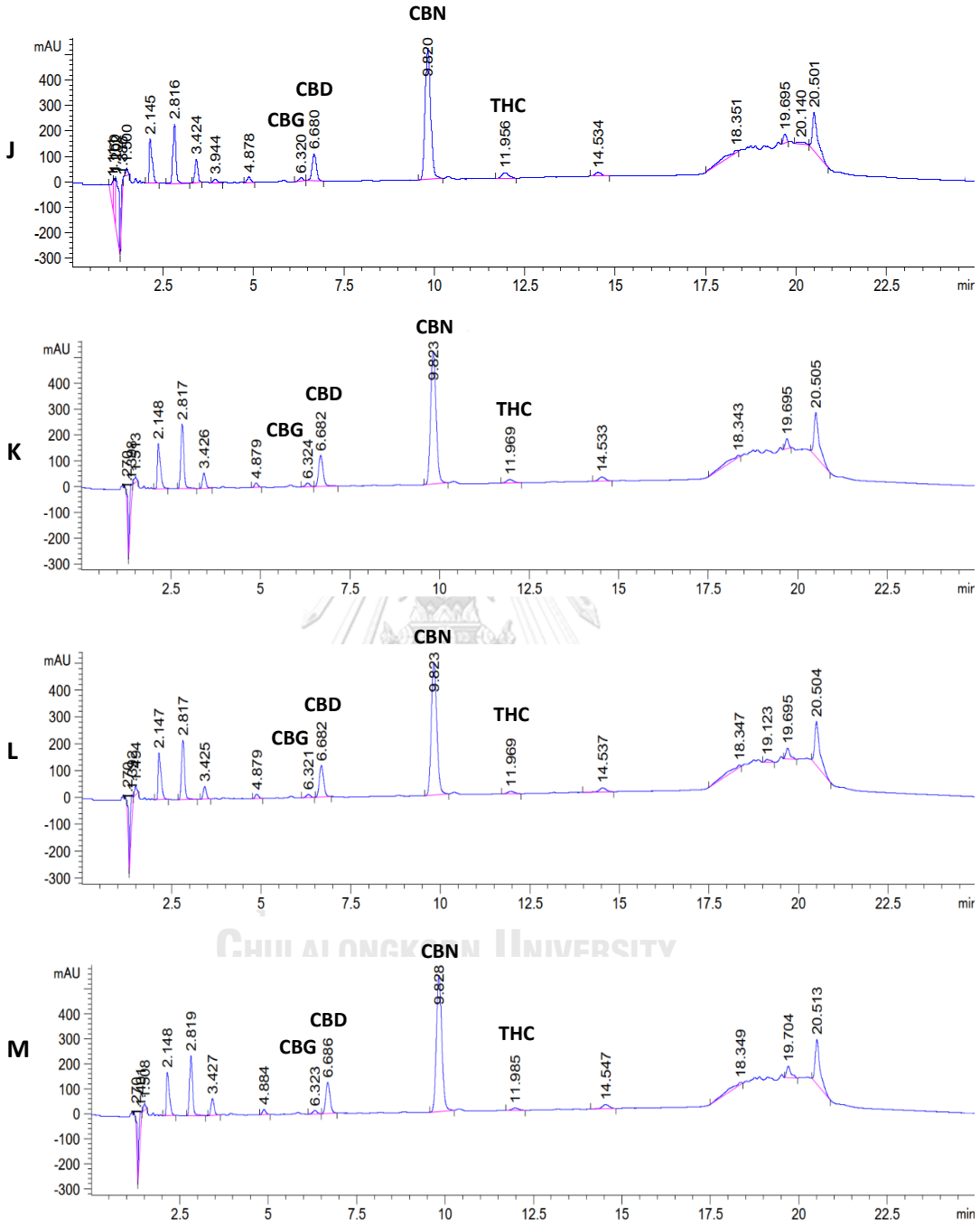
(F) ethanol; (G) hexane

HPLC chromatogram of *C. sativa* strain CBD Charlotte's Angel female flower vegetable oil extracts (decarboxylated sample)

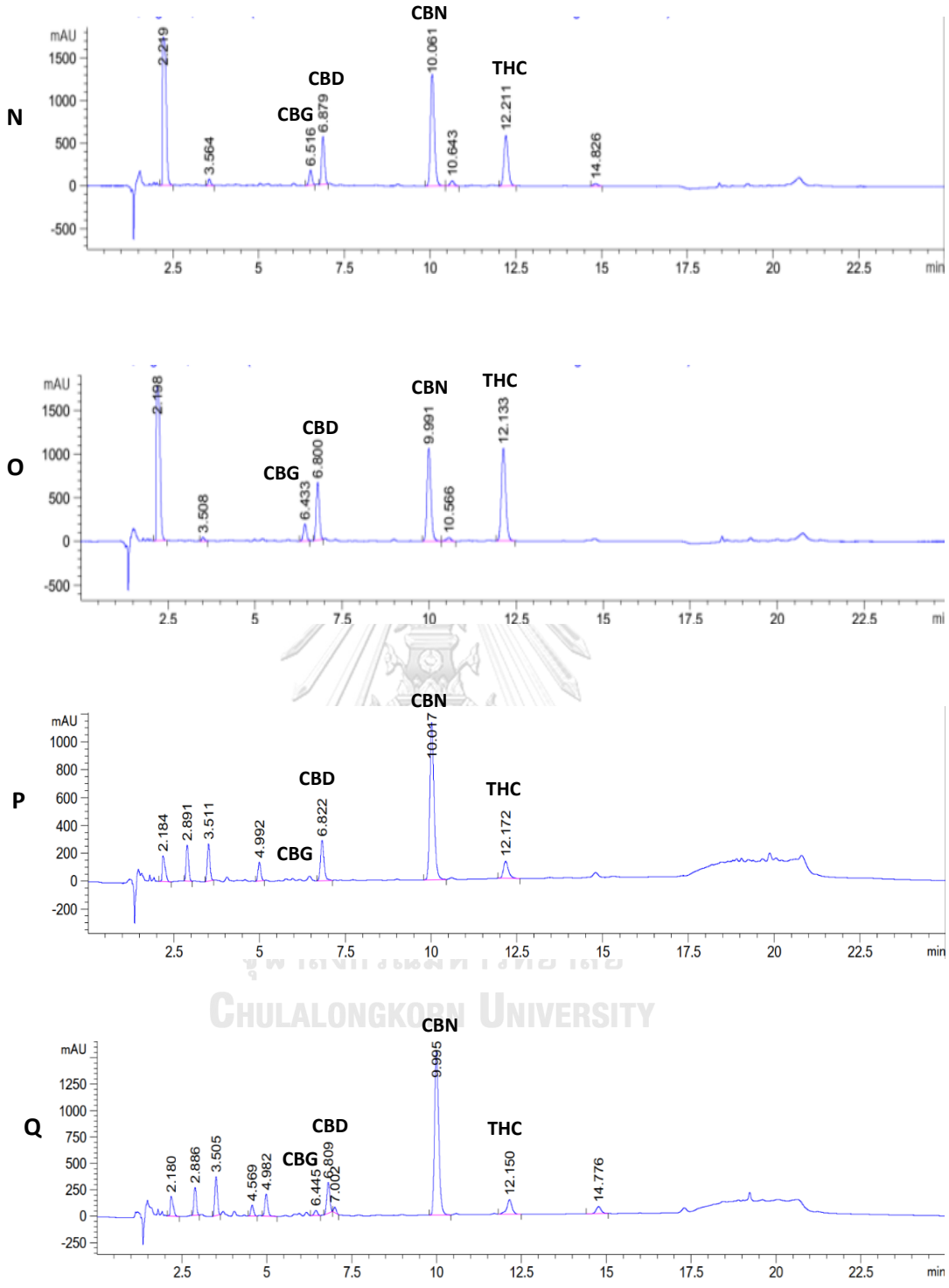


(H) virgin coconut oil; (I) olive oil

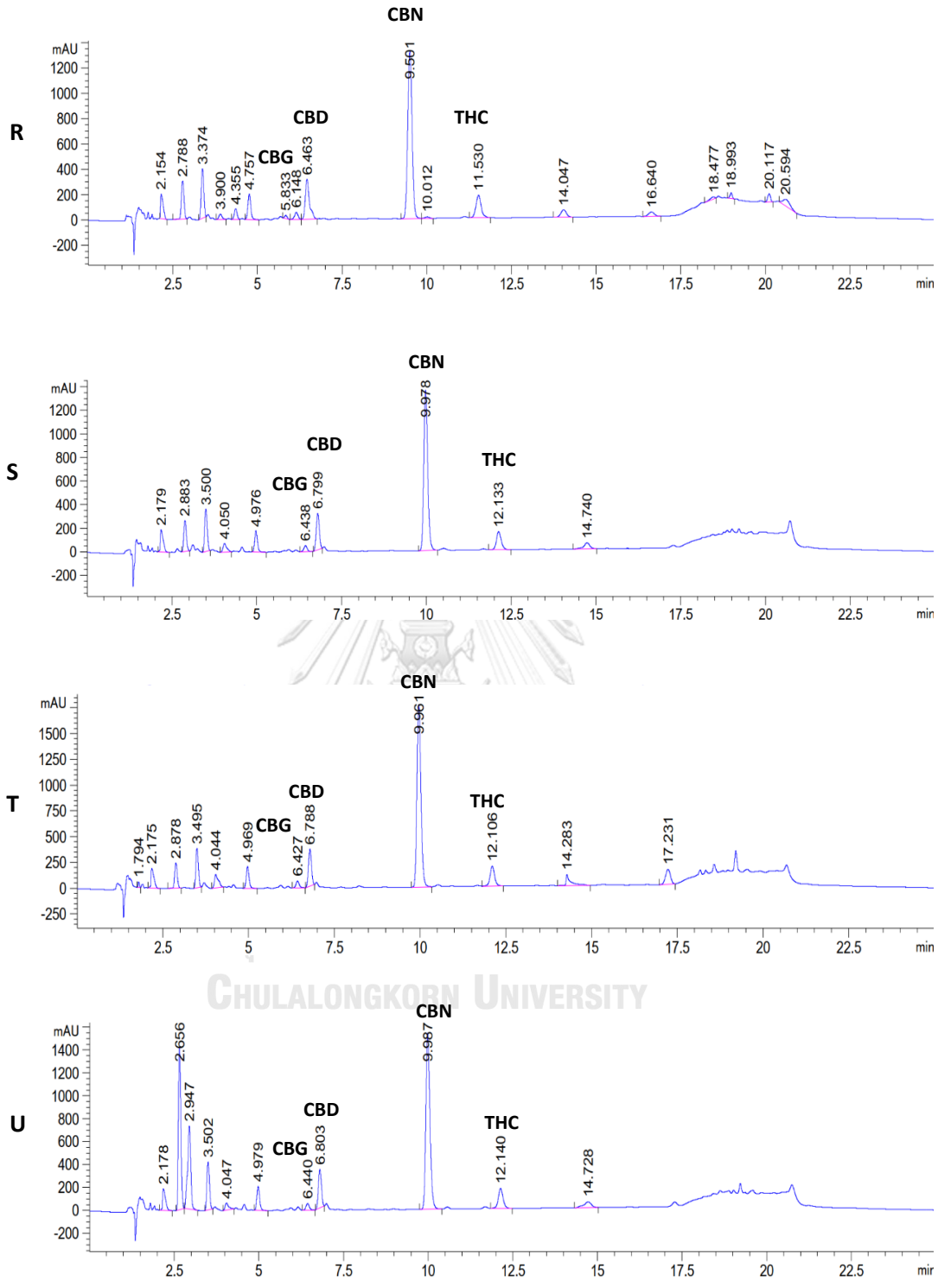
HPLC chromatogram of *C. sativa* L. obtained from ONCB Thailand



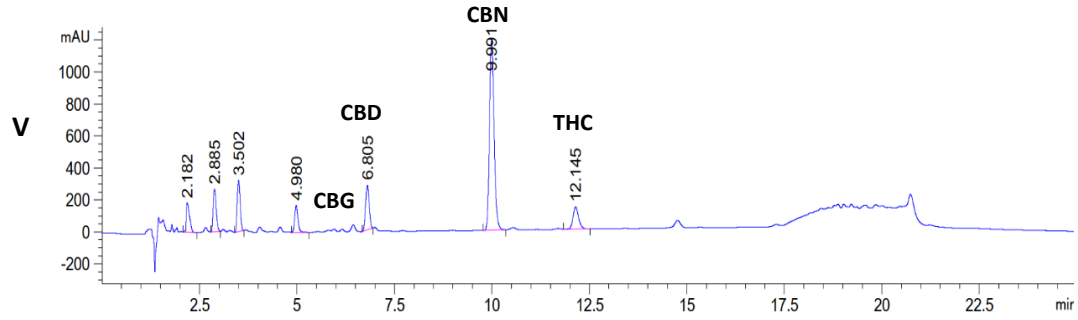
(J) ethanol; (K) hexane; (L) light petroleum; (M) heavy petroleum



(N) $SCCO_2$; (O) W-$SCCO_2$; (P) virgin coconut oil; (Q) RF-olive oil



(R) HH-olive oil; (S) sacha inchi oil; (T) rice bran oil; (U) sesame seed oil



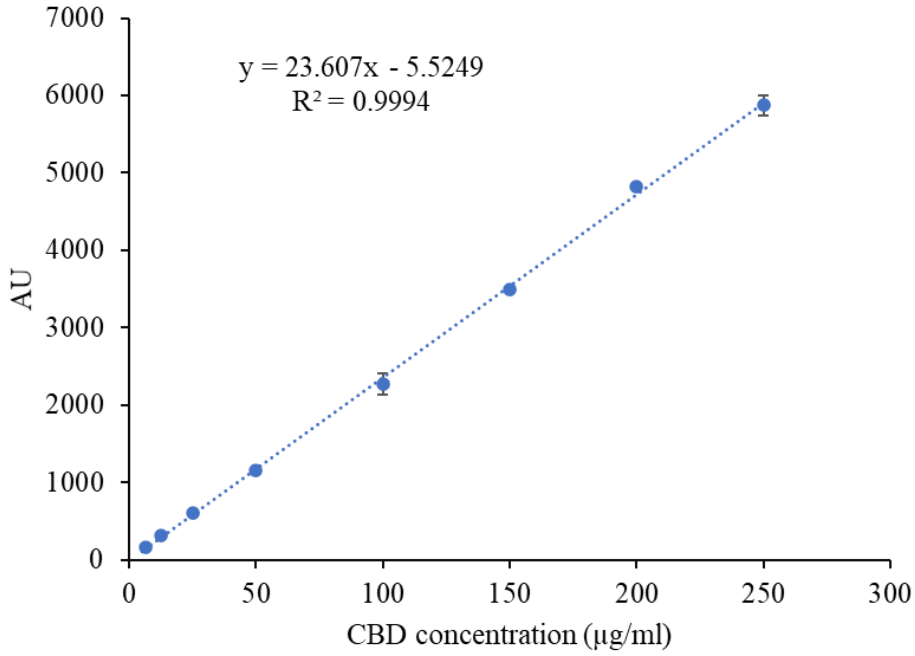
(V) perilla seed oil

Cannabinoids standard for HPLC analysis

Cannabidiol (CBD)

Cons (µg/mL)	6.25	12.5	25	50	100	150	200	250
	158.62448	312.0369	612.5732	1164.572	2352.069	3515.615	4836.203	5732.723
	158.39241	311.4999	607.8836	1158.834	2113.063	3483.45	4817.204	5906.27
	158.69301	309.1883	606.8139	1159.61	2337.966	3494.591	4797.789	5976.429
Δ	158.569967	310.9084	609.0903	1161.005	2267.699	3497.885	4817.065	5871.807
σ	0.15754005	1.513645	3.063411	3.113065	134.1042	16.33358	19.20717	125.4548
% RSD	0.0993505	0.486846	0.502949	0.268135	5.913665	0.466956	0.398732	2.136562

Λ = 228 nm

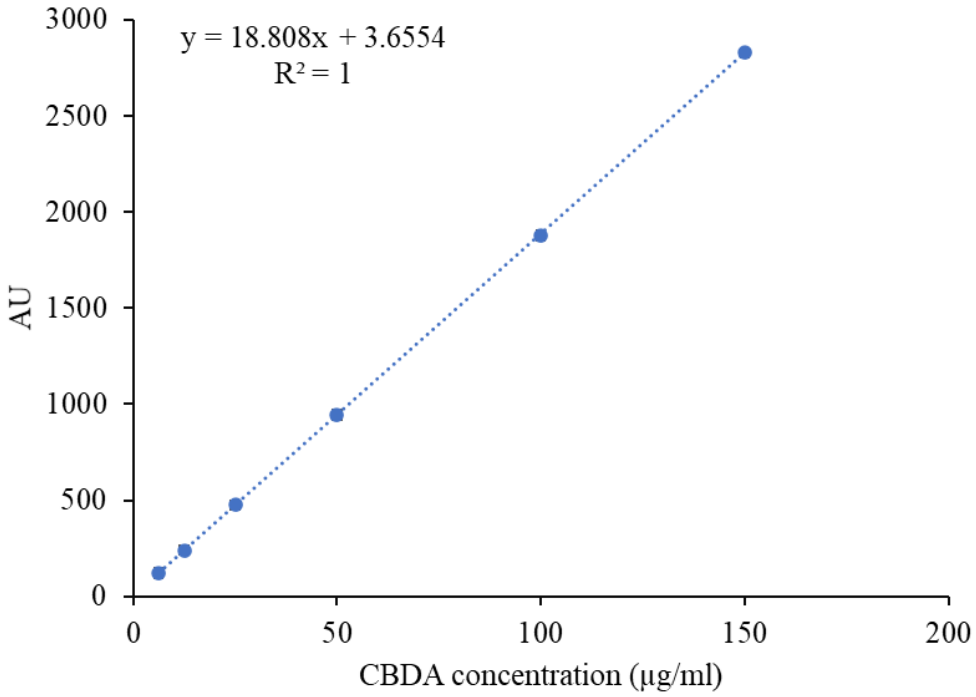


Cannabinoids standard for HPLC analysis

Cannabidiolic acid (CBDA)

Cons (µg/mL)	6.25	12.5	25	50	100	150	6.25
	120.12083	238.7595	476.7406			2829.143	120.12083
	120.7192	240.7089	476.3996	943.9501	1882.324	2827.582	120.7192
	120.1214	240.6859	473.8746	945.0676	1873.832	2829.224	120.1214
Δ	120.320477	240.0514	475.6716	944.5088	1878.078	2828.65	120.320477
σ	0.34530465	1.118909	1.565584	0.790192	6.004539	0.925549	0.34530465
% RSD	0.28698744	0.466112	0.329131	0.083662	0.319717	0.032721	0.28698744

Λ = 270 nm

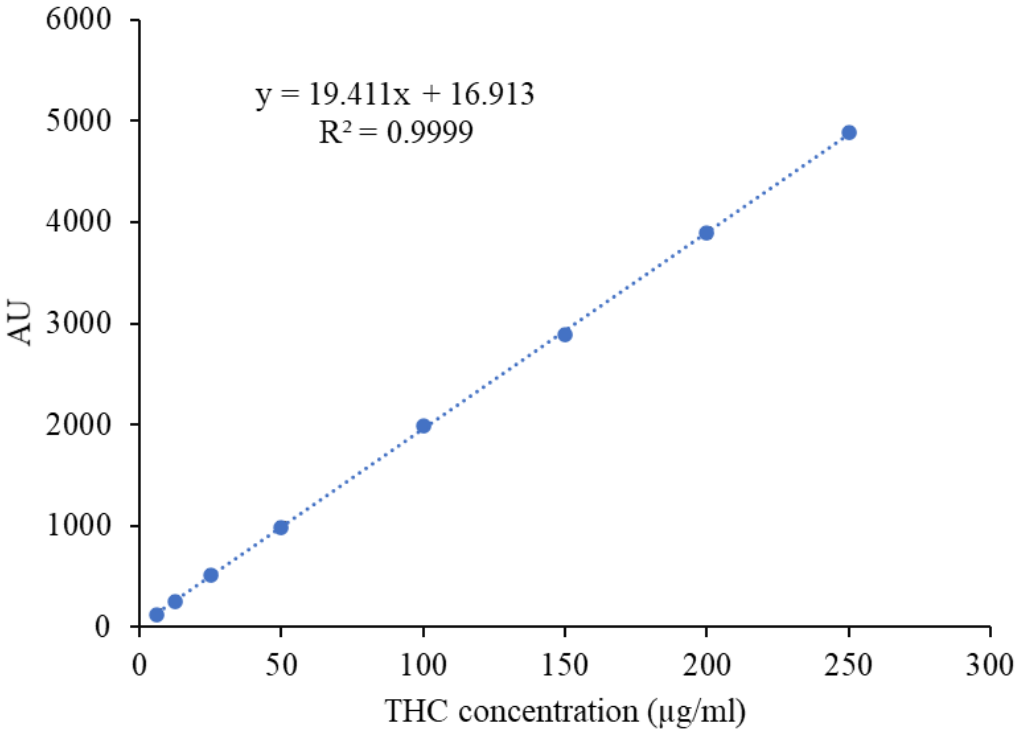


Cannabinoids standard for HPLC analysis

Δ^9 – Tetrahydrocannabinol (THC)

	6.25	12.5	25	50	100	150	200	250
Cons (µg/mL)	127.65527	258.1429	513.9652	1002.033	1980.343	2842.706	3915.913	4867.638
	127.37639	258.752	513.4854	994.8914	1982.131	2920.837	3866.856	4879.795
	128.23503	260.5877	513.4303	966.7604	1993.156	2906.813	3898.786	4907.513
Δ	127.755563	259.1609	513.627	987.8949	1985.21	2890.119	3893.852	4884.982
σ	0.43801795	1.272668	0.29418	18.64801	6.939571	41.65493	24.89819	20.43726
% RSD	0.34285626	0.491073	0.057275	1.887651	0.349564	1.441288	0.639423	0.418369

Λ = 270 nm



VITA

NAME	NUR ALFAN MUHAMMAD ZEN
INSTITUTIONS ATTENDED	Sam Ratulangi University Indonesia
PUBLICATION	Bioactivity Testing of Ethanol Extract of Padina australis from Molas Beach North Sulawesi against Staphylococcus epidermidis (2015)