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Vegetable Oil-Based Cannabis: Its Cannabinoid Profiling and Photoprotective Effect on UVA-Irradiated Human Skin Keratinocytes

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ABSTRACT

Vegetable oils serve as affordable, non-toxic, and eco-friendly solvents for plant extraction. Chemical fingerprinting shows that vegetable oil-based cannabis has a cannabinoid profile similar to that of organic solvent-based cannabis. All samples possess antioxidative effects as they contain several cannabinoids and other unidentified compounds, with rice bran oil-based sample exhibiting the strongest effect. Tetrahydrocannabinol (THC) and cannabidiol (CBD) content in sesame oil-based and perilla seed oil-based cannabis differed significantly from that in virgin coconut oil (VCO)-based cannabis. In addition, THC and CBD content in all vegetable oil-based samples prepared using the heating and frying method differed significantly from that in samples prepared using conventional methods. The VCO-based cannabis was chosen to preliminarily test its photoprotective effect and antioxidant enzyme activity. The results showed that it can protect UVA-irradiated HaCaT cells. Pretreatment with VCO-based cannabis can promote Nrf-2/HO-1 signaling, thereby upregulating antioxidant enzymes and increasing the capacity of keratinocytes to detoxify oxidative insults. Herein, we present the first overview of the cannabinoid profile of vegetable oil-based cannabis, which can be used for quality control during the development of vegetable oil-based cannabis products. Furthermore, the skin photoprotective properties of oil-based extracts can provide supporting evidence for the formulation of cannabis-based skincare products.

Keywords: Antioxidant, cannabidiol, cannabis, cosmeceutical, tetrahydrocannabinol, virgin coconut oil

INTRODUCTION

Sunlight is the best source of Vitamin D for humans; however, it can have problematic effects, especially on the skin. The skin primarily functions as a protective barrier between the body and external environment. Specifically, the skin structure is arranged in superimposed layers that serve as an effective outer biological barrier.[1] Long-term exposure to sunlight can cause sunburn, chronic skin damage (e.g., photoaging), or even skin cancer. Excessive exposure to sunlight can also cause ocular diseases (including cataracts and pterygia).[2] It is known that sunlight's ultraviolet B

(UVB) radiation, with a wavelength (λ) of 280–320 nm, can penetrate the human skin (up to a depth of 160−180 μm), leading to oxidative stress, skin pigmentation, DNA damage, immunosuppression, hyperplasia, skin cancer, erythema, and sunburn.[3-6] However, sunlight's ultraviolet A (UVA) radiation, with a λ of 320–400 nm, can penetrate the skin even deeper than UVB, accelerating free radical formation and damaging DNA, proteins, and lipids.[3,4,6-9] The skin absorbs UVA photons deeply into the epidermis and dermis. Approximately 80% of the UVA radiation can reach the dermoepidermal junction and papillary dermis. A majority of skin cells (including endothelial cells in blood vessels, keratinocytes, fibroblasts, and melanocytes) can be affected by UVA radiation.[10]

The demand for the development and exploration of alternative and potent skin photoprotectants is ever-increasing. Several inorganic compounds conventionally used as sunscreen ingredients (such as zinc oxide, titanium dioxide, and other minerals) have been proven to reflect and scatter both UVA and UVB radiation. However, concerns regarding their safety and potential toxicity have been raised, as these metal-based compounds often form an unwanted opaque layer on the skin.[11] Thus, extensive research on bioactive compounds derived from natural sources is needed. Plants are known to produce photoprotective compounds; therefore, these compounds could replace synthetic ingredients in cosmeceutical products or minimize our dependence on them. It should be kept in mind that UV filters are characterized by low photostability and poor ability to protect against a narrow λ spectrum; this is why the implementation of herbal antioxidants (phytoantioxidants) in UV-filtering cosmetics might be a useful strategy.[12] The enhancement of the skin's capacity to neutralize reactive oxygen species (ROS), which can be generated both endogenously as well as by an external factor (UV radiation), makes antioxidants a promising solution in this respect.[13]

Cannabis sativa L. (widely known as marijuana or cannabis) is a plant that produces a unique class of terpeno-phenolic compounds (cannabinoids); to date, 120 of 565 constituents of this plant have been isolated and are collectively known as phytocannabinoids.[14] Over the last century, the ban on cannabis use has been gradually lifted as many countries consider Δ^9-tetrahydrocannabinol (THC) as a therapeutic agent. *C. sativa* products may have therapeutic applications in the treatment of various diseases, including sleep disorders, posttraumatic stress disorder, and Tourette's syndrome.[15-17] The ongoing research on the medical applications of this plant is facilitated by an increase in public interest for *C. sativa*; thus, it is necessary to focus on research related to the phytochemical composition of this plant and its products as well as on extraction methods for the isolation of its bioactive compounds.[18] Temperature and solvent type are two important factors that highly affect the quality of extraction. Exposing the solvent to high temperatures can facilitate the extraction process as it can break the cell wall, releasing bioactive compounds from the cells. However, high temperatures can degrade certain thermolabile compounds.[19] Dichloromethane, ethanol, trimethylpentane, and chloroform are some of the commonly used organic solvents in cannabis extraction performed using the maceration method.[20]

To date, supercritical fluid extraction (SFE) has been one of the most common methods for cannabinoid extraction; SFE is particularly preferable in terms of operation economy, environmental concerns, and large-scale purification technicalities.[21,22] Solvents in the chemical industry are used in massive quantities. Hence, the required costs and implicated health and safety concerns are integral and highly impactful aspects of solvent selection. Green solvents are an innovation that is suitable for reducing the environmental impact of the solvent production process.[23] In fact, several studies have already applied innovative techniques (such as the use of vegetable oils as solvents and cosolvents) in the extraction of various bioactive compounds.[24-27]

It is undeniable that in recent years, the interest in using vegetable oil as a solvent for plant compound extraction (including *C. sativa*) has increased, as several studies have reported the use of olive oil as a solvent in cannabis extraction.[28,29] Extraction using coconut oil has also been developed and applied in Thailand by the Department of Thai Traditional and Alternative Medicine of the Ministry of Public Health of Thailand. Coconut oil is used in the development of the Deja formula (ganja oil), which uses the method of heating and frying (H and F method) the flower of *C. sativa* in this oil.[30] Despite these developments, our knowledge of the chemical profile of vegetable oils used *C. sativa* extraction and of the antioxidant activity of these extracts is still lacking. At present, *C. sativa* extraction is limited to a few types of oils (such as olive oil, sunflower oil, and coconut oil);[31] however, several other vegetable oils that are commonly used in the household are currently available on the market. This study therefore aimed to investigate the effects of various vegetable oils (compared with common solvents) on cannabinoid extraction and evaluate the antioxidant properties of these extracts as well as their ability to protect human skin keratinocytes from UVA radiation *in vitro*.

MATERIALS AND METHODS

Materials

C. sativa was supplied by the Office of the Narcotics Control Board (ONCB) of Thailand. It was ground into powder and subsequently decarboxylated by heating the material at 110°C for 60 min in a hot-air oven.[32] Vegetable oils used as solvents in this study included virgin coconut oil (VCO) (Plearn®, Thailand), sacha inchi oil (SIO) (BioTrade Thai, Thailand), perilla seed oil (PSO), sesame seed oil (SSO), olive oil of roasting and frying grade (RF-olive oil) (Bertolli®, Spain), olive oil of high-heat cooking grade (HH-olive oil) (Bertolli®, Spain), and rice bran oil (RBO). The organic solvents used for extraction included ethanol, light petroleum ether (boiling point: 40−60°C), heavy petroleum ether (boiling point: 60−80°C), and hexane from Sigma-Aldrich®, USA. Other chemicals used in this study included methanol, chloroform, vanillin, sulfuric acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH); all the above-mentioned solvents and chemicals were purchased from Sigma-Aldrich®, USA. The cannabidiol-rich cannabis extracts (CRCE) contained cannabidiol (CBD) distillate broad-spectrum 0% THC purchased from CBD Capital Ltd., Surrey, UK (Cat No. CBD102). The cannabinoid reference standards used were cannabidiolic acid (CBDA), cannabigerol

(CBG), tetrahydrocannabinolic acid (THCA), THC (Cayman Chemical Co, USA), cannabinol (CBN), and CBD (THC Pharm GmbH, Germany). Human skin keratinocytes (HaCaT cell line) used for photoprotective assays were obtained from Elabscience (Houston, TX, USA).

Methods

Sample extraction and preparation

Five grams of the decarboxylated cannabis powder (preheated to 110°C for 30 min) was macerated using various organic solvents and vegetable oils, with a ratio of cannabis powder to solvent/oils of 1:10, for 24 h. Extracts obtained from organic solvent extraction were then filtered using Whatman paper No. 1 and evaporated using a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) to obtain crude extracts. Vegetable oil-based samples were collected and filtered to obtain final oil samples with a concentration of 100 mg/mL as stock solution. For SFE, 60 g of cannabis powder was added to the extraction vessel, and the extraction was executed at 4 h, 55°C, and pressure of 225 bar using CO_2 as solvent. At the end of the process, the powder was collected from the extraction vessel and then 95% ethanol was used to rinse off the remnants in the pipes for 30 min at a rate of 1 mL/min (CRS Supercritical Fluid Extractor R401, Korea). An aliquot of the supercritical CO_2 ($ScCO_2$) extract was mixed with ethanol (1:10 w/v) and this mixture was cooled to −20°C overnight. Finally, it was filtered and evaporated to obtain the winterized supercritical CO_2 (W-$ScCO_2$) extract. Simultaneously, another 5 g of the decarboxylated cannabis powder was fried in vegetable oils (VCO, RBO, olive oil, PSO, SIO, and SSO) for 30 min at 250°C. Then, all the fried powder materials were re-soaked for 24 h in the respective vegetable oils and the supernatant was collected and filtered to obtain H and F samples for THC and CBD quantification.

Samples were further prepared for cannabinoid analysis and DPPH-radical scavenging assay. Briefly, 1 mg crude extracts of organic solvents were dissolved in the solvent mixture (methanol: chloroform; 9:1), sonicated for 30 min, and centrifuged at 10,000 rpm for 5 min to obtain 1 mg/mL organic solvent extract solution, while 200 µL of 100 mg/mL of each vegetable oil-based cannabis stock solution was dissolved in 800 µL of methanol and chloroform (9:1), sonicated for 30 min, and centrifuged at 10,000 rpm for 5 min to obtain 20 mg/mL vegetable oil-based cannabis solution.

Cannabinoid profiling and quantification

High-performance thin-layer chromatography (HPTLC) was used to determine the cannabinoid profile of the samples; 5 µL of each sample and 4 µL of the cannabinoid standard (100−200 ng/mL) were applied on silica gel 60 F_{254} HPTLC glass plates (10 × 20 cm; Merck, Germany) using the applicator CAMAG Linomat 5 (CAMAG, Switzerland). The development system used was a heptane: diethyl ether: formic acid (90:20:0.3 v/v/v) mixture. The plates were developed to 70 mm using an automatic developing chamber (CAMAG, Switzerland), while images were acquired using the TLC Visualizer (CAMAG, Switzerland), which captured images under white light and UV light (at 254 and 366 nm). Image scanning and analysis were performed using the VisionCATS software (CAMAG, Switzerland). The plates were then subjected to postchromatographic derivatization using 1% vanillin reagent in ethanol and heated to 105°C on a hot plate.

For the quantification of THC and CBD, HPLC analysis was performed as described previously,[33] with slight modifications. All samples were analyzed using the Agilent 1260 Infinity II HPLC system (Agilent Technologies, USA) that included a flexible pump, a vial sampler, a thermostat column compartment, and a diode array detector. An EC-C18 column guard (3.0 × 5 mm, 2.7 µm, InfinityLab Poroshell 110, Agilent) and a reverse phase EC-C18 column (3.0 × 150 mm, 2.7 µm, InfinityLab Poroshell 110, Agilent) were used. The column temperature was set at 35°C, and the gradient elution used in this method was 0.1% formic acid in water (A) and acetonitrile (B), with a flow rate of 0.5 mL/min for 25 min. The gradient conditions were as follows: 0−15.0 min, 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; and 18.1−25.0 min, 70% B. The injection volume used was 5 µL. The detection wavelength used for THC and CBD was 228 nm.

DPPH-radical scavenging assay

The free radical scavenging capacity of each sample was examined using a slightly modified method of Dolly *et al.*[34] Briefly, 75 µL of the freshly prepared DPPH solution in methanol (0.4 mM) was carefully introduced into each well of a 96-well microplate. Subsequently, 50 µL of the prepared organic solvent and vegetable oil-based cannabis samples as well as 1 mg/mL of gallic acid standard in various concentrations of methanol were added to each well. A blank containing methanol and DPPH was used. The microplate was left in the dark for 30 min. Thereafter, the plate was placed in a microplate reader (CLARIO Star microplate reader, BMG Labtech, Germany) and the absorbance was measured at 517 nm. The assay was performed in triplicates and the percentage inhibition of each sample or standard was calculated using the following equation:

$$\%Inhibition = \frac{OD_{blank} - OD_{sample}}{OD_{blank}} \times 100$$

TLC–DPPH bioautography assay

The HPTLC plates that were developed (described in section 2.2.2) were dried and then sprayed with 0.2% DPPH in methanol. Shortly thereafter, the plates were incubated at 25°C for 30 min in a dark room. Images were visualized using CAMAG TLC Visualizer 2 (CAMAG, Switzerland).[35]

Cell culture

HaCaT keratinocytes (EP-CL-0090; Elabscience, TX, USA) were cultured in Dulbecco's modified Eagle medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). The cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

Preparation of VCO-based Cannabis and $ScCO_2$ Sample for the cell-based assay

The VCO-based cannabis and $ScCO_{2\ sample}$ of cannabis were chosen in this experiment, and their THC and CBD contents were standardized using the amount of THC quantified by the HPLC method. Samples were dissolved in DMSO (solvent) to produce 1 mg/mL of THC and CBD stock solution each. To evaluate the photoprotective activity against UVA radiation, cells were treated with the samples (THC, final concentration: 155–1250 ng/mL in 0.5% DMSO, a nontoxic concentration of DMSO; CBD, final concentration: 155–1250 ng/mL in 0.5% DMSO) for 18 h before UVA irradiation. Control cells were incubated with 0.5% of an equivalent amount of DMSO.

UVA irradiation

After the aforementioned pretreatments, the cells were gently washed twice with ice-cold phosphate-buffered saline (PBS), and a serum-free medium was added. Cells were then irradiated with UVA radiation (using a UVA light bulb at 365 nm; 7 J/cm^2) for 35 min using a UVP crosslinker CL-3000L (Analytik Jena, Jena, Germany). Before the irradiation experiments, the experimental medium was aspirated and ice-cold PBS was added. To ensure the uniformity of irradiation in each experiment, culture plates were placed in the middle of the machine at a similar position. During irradiation, cells were directly exposed to UVA, that is, without the culture lid. After exposure to UVA, the experimental medium was aspirated, and the cells were washed twice with PBS, cultured in a regular medium for 24 h, and then assessed for their viability or expression of indicated proteins.

Evaluation of cell viability

The viability of cells was assessed through a conventional MTT assay.[36] Briefly, HaCaT cells were seeded into 96-well culture plates (2×10^4 cells per well in 200 µL medium) and cultured for 24 h. After the application of the indicated experimental conditions, the medium was finally removed. Cells were gently washed with PBS and subsequently incubated with a solution of MTT (1 mg/mL in serum-free DMEM; 200 µL) for 3 h in the dark. After incubation, the MTT solution was discarded and the insoluble formazan crystal was dissolved into 200 µL of DMSO. The absorbance of the formazan solution was measured at 570 nm using a CLARIOstar microplate reader (BMG Labtech, Germany).

Western blot analysis

After the application of the indicated experimental conditions, HaCaT cells were lysed with ice-cold RIPA buffer (Cell Signaling, Danvers, MA, USA; cat. no. 9806). Equal amounts of protein lysates were fractionated on 10% SDS–PAGE and electrotransferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories Inc., Hercules, CA, USA). The membranes were blocked with 5% nonfat milk and incubated with the primary antibody overnight at 4°C; the following primary antibodies were used: anti-Nrf2 (antibody against nuclear factor-erythroid 2 related factor 2; 1:1000; Abcam, Cambridge, MA, USA; ab137550), anti-GPx1 (antibody against glutathione peroxidase-1; 1:1000; Abcam; ab22604), anti-CAT (antibody against catalase; 1:1000; Cell Signaling; cat. no. 12980), anti-HO-1 (antibody against heme oxygenase-1; 1:1000; Cell Signaling; cat. no. 43966), and anti-GAPDH (antibody against glyceraldehyde-3-phosphate dehydrogenase; 1:2000; Cell Signaling; cat. no. 5174). Incubation with secondary antibodies (antirabbit IgG, HRP-linked antibody; 1:2000; Cell Signaling; cat. no. 7074) was performed at 25°C for 1 h. The signal was developed using Immobilon Western chemiluminescent HRP substrate (EMD Millipore, Burlington, MA, USA; cat. no. P90719) and visualized using an ImageQuantTM LAS 4000 biomolecular imager (GE Healthcare, Chicago, IL, USA). The band intensities of proteins were quantified using the ImageJ software.

Statistical analysis

Independent *t*-test, one-way analysis of variance (ANOVA) followed by Tukey's *post hoc*, and Dunnet multiple comparisons were performed to examine statistical differences between the recorded means. All means and standard error of means (SEMs) were calculated from three independent experiments. Statistical analyses were performed using GraphPad Prism (version 9.3.1).

RESULTS

Cannabinoid Profiling of Vegetable Oil-Based Cannabis

The target cannabinoids in the samples demonstrated good separation in the heptane: diethyl ether: formic acid (90:20:0.3 v/v/v) mobile phase mixture on the HPTLC plate. After visualizing the plate under 254-nm and 366-nm UV light and white light as well as after subjecting it to derivatization using 1% vanillin reagent, the white light post derivatization resulted in the best visual separation of the studied compounds [Figure 1]. The results of the cannabinoid profiling confirmed the presence of several cannabinoids with identical colors in each compound (after derivatization), including CBD (dark red), THC (brown), CBN (rosette), and CBG (dark orange). CBD and CBN were detected very strongly in all samples. Conversely, CBG and THC were detected very weakly in all samples, except ethanol and hexane samples, in which they were detected slightly more intensely. Four bands (top) were detected in all samples (organic solvent extract and vegetable oil-based cannabis), indicating the presence of CBD, THC, CBN, and CBG (R_f: 0.35–0.5). Several bands of the vegetable oil-based cannabis (such as those of RBO-, SIO-, SSO-, and PSO-based cananbis) were characterized by tailed bands, except for those of the HH- and RF-olive oil. Some unidentified bands (indicating other compounds in the extract) were also detected in all samples (at R_f: = 0.1–0.2 and R_f: = 0.2–0.3) of the vegetable oil-based cannabis.

Quantification of Cannabinoids in Vegetable Oil-based Cannabis

To determine the effect of the chosen vegetable oil type as a solvent on the THC and CBD content of the samples, quantification was performed via HPLC and was statistically analyzed using one-way ANOVA (in which the VCO acted as the control group) [Figure 2a]. The results demonstrated that there was no significant difference in THC and CBD contents between the VCO-based cannabis and several other vegetable

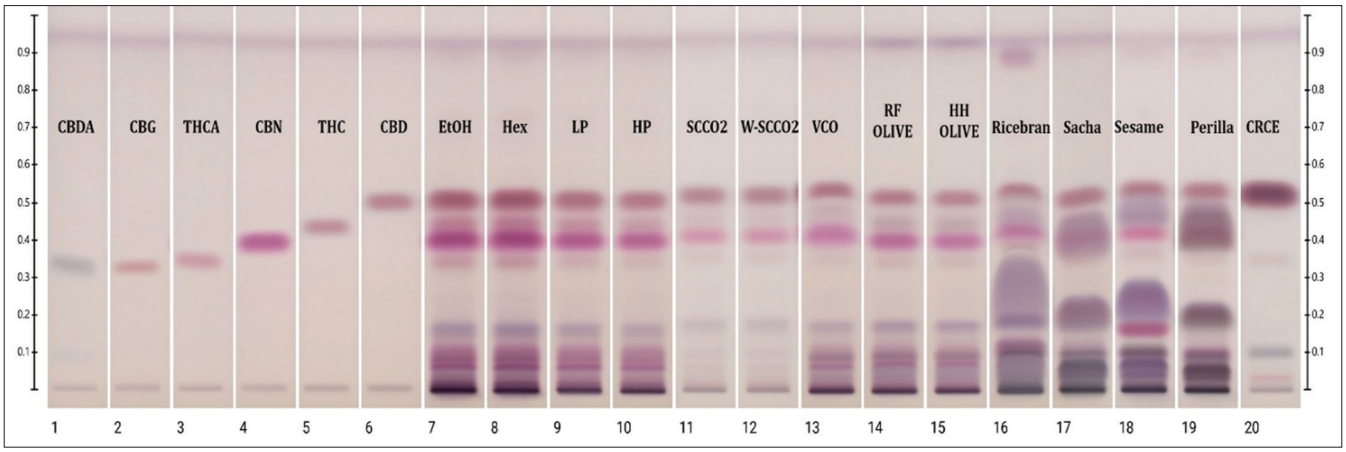

Figure 1: High-performance thin-layer chromatography chemical profile of organic solvents extracts, $ScCO_2$ extracts, and vegetable oil-based cannabis. Images taken at white light after derivatization with 1% vanillin reagent; track 1: Cannabidiolic acid, track 2: Cannabigerol, track 3: Tetrahydrocannabinolic acid, track 4: Cannabinol, track 5: Tetrahydrocannabinol, track 6: Cannabidiol, track 7: Ethanol, track 8: Hexane, track 9: Light petroleum ether, track 10: Heavy petroleum ether, track 11: $ScCO_2$, track 12: W-$SCCO_2$, track 13: Virgin coconut oil, track 14: Roasting and frying olive oil, track 15: High-heat olive oil, track 16: Rice bran oil, track 17: Sacha inchi oil, track 18: Sesame oil, track 19: Perilla seed oil, and Track 20: Cannabidiol distillate

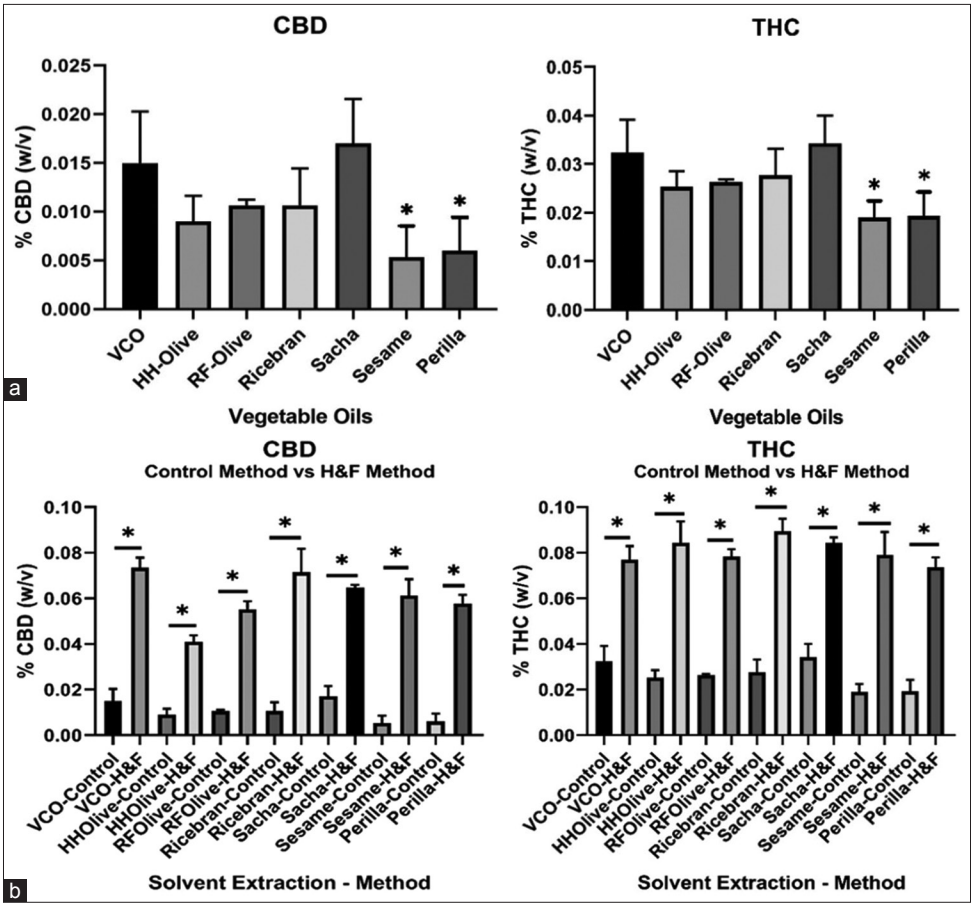

Figure 2: (a) Tetrahydrocannabinol and cannabidiol content of vegetable oil-based cannabis. Vertical bars indicate standard deviation (n = 3); *represents significant differences (vegetable oil vs. virgin coconut oil-based cannabis as a control) at $P < 0.05$, as calculated by one-way ANOVA followed by the Dunnett multiple comparison test. (b) Effect of the applied extraction method on Tetrahydrocannabinol and cannabidiol contents. All experiments were conducted in triplicate (n = 3); *Represents significant differences (modified method vs. control method) at $P < 0.05$, as calculated by independent t-tests

oil-based cannabis samples, except for the SSO- and PSO-based cannabis, which showed a significantly lower content ($P < 0.05$) of these compounds than the VCO-based cannabis. The SIO-based cannabis exhibited the highest content of THC

(0.034 ± 0.006% w/v) and CBD (0.017 ± 0.005% w/v), followed by the VCO-based cannabis (THC: 0.033 ± 0.007% w/v and CBD: 0.015 ± 0.005% w/v).

In addition, the effect of extraction methods on the THC and CBD content of the samples was compared between the cold maceration (as a conventional/control method) and H and F method (as a modified method). The results of the independent *t*-test revealed that the H and F method resulted in a significantly higher THC and CBD content in all samples than the cold maceration method [Figure 2b]. The THC content of the samples treated by the H and F method did not differ significantly among the studied samples, except for the VCO-based cannabis when compared with the RBO-based cannabis. Conversely, the CBD content differed significantly ($P < 0.05$) among the studied H and F samples, except for the VCO-based cannabis when compared with the RBO-based cannabis. The RBO-based cannabis had the highest content of THC (0.090 ± 0.005% w/v), whereas the VCO-based cannabis had the highest content of CBD (0.073 ± 0.005% w/v).

DPPH-Radical Scavenging Assay

The antioxidant activities of cannabis extracted using various organic solvents, $ScCO_2$, and vegetable oils were assessed through a DPPH-radical scavenging assay. Table 1 presents the IC_{50} values of each sample. Among all organic solvent extracts tested, the W-$ScCO_2$ demonstrated the most potent radical scavenging activity (0.030 ± 0.006 mg/mL), followed by hexane (0.048 ± 0.003 mg/mL), $ScCO_2$ (0.066 ± 0.004 mg/mL), ethanol (0.079 ± 0.039 mg/mL), light petroleum ether (0.136 ± 0.017 mg/mL), and heavy petroleum ether (0.348 ± 0.022 mg/mL,). Among vegetable oils, RBO-based cannabis had the strongest potency in scavenging DPPH free radicals, with an IC_{50} value of 3.345 ± 0.120 mg/mL, followed by VCO-based cannabis (5.812 ± 1.396 mg/mL), SIO-based (7.050 ± 0.081 mg/mL), HH-olive-oil-based cannabis (7.663 ± 0.753 mg/mL), RF-olive-oil-based cannabis (11.878 ± 0.375 mg/mL), PSO-based cannabis (13.137 ± 1.062 mg/mL), and SSO-based cannabis (13.696 ± 0.565 mg/mL). The antioxidant abilities of HH-olive-oil- and SIO-based cannabis did not differ significantly from that of VCO-based cannabis (control), whereas those of RF-olive-oil-, PSO-, RBO-, and SSO-based cannabis differed significantly from that of VCO-based cannabis ($P < 0.05$).

Table 1: DPPH-radical scavenging activity of organic solvent extracts and vegetable oil-based extracts (mean±SD)

Sample	DPPH IC_{50} (mg/mL)
Gallic acid	0.0089±0.0005
Organic solvent extract	
W-$ScCO_2$	0.030±0.006
Hexane	0.048±0.003
ScCO2	0.066±0.004
Ethanol	0.079±0.039
Light petroleum	0.136±0.017
Heavy petroleum	0.348±0.022
Vegetable oil-based cannabis	
Rice bran oil	3.345±0.120*
Virgin coconut oil	5.812±1.396
Sacha Inchi oil	7.050±0.081
HH-olive oil	7.663±0.753
RF-olive oil	11.878±0.375****
Perilla seed oil	13.137±1.062****
Sesame seed oil	13.696±0.565****

HH: High-heat cooking grade; RF: Roasting and frying grade; (*): significant differences; *$P<0.05$; ****$P<0.0001$ (vegetable oil-based cannabis vs. virgin coconut oil-based cannabis). DPPH: 2,2-diphenyl-1-picrylhydrazyl

TLC–DPPH

The TLC–DPPH screening method revealed the presence of antioxidant compounds in all tested samples. The TLC plates that were developed were sprayed with 0.2% DPPH reagent (diluted in methanol). The appearance of a yellow band on a purple background indicated antioxidant activity. Figure 3 presents the TLC–DPPH chromatogram after spraying with the DPPH reagent. Cannabinoid reference standards were tested along with the extracts. Among the six standard compounds tested, CBDA and THCA demonstrated weak antioxidant activity, whereas CBN, CBD, CBG, and THC demonstrated strong antioxidant activity. All tested samples exhibited many bands, thereby indicating their antioxidant activity. CBG, CBN, THC, and CBD found in the extracts clearly contributed to the overall antioxidant activity; however, other unidentified compounds could have also contributed to the overall antioxidant activity. Both organic solvent extracts and vegetable oil-based cannabis extracts revealed CBN and CBD, followed by THC, as the most potent sources of antioxidant activity.

Cytotoxicity of the VCO-Based Cannabis and $ScCO_2$ Cannabis Samples on Human Skin Keratinocytes

The cannabis sample used in this study was obtained from a $ScCO_2$ extraction, which is the most commonly used method to extract cannabis. VCO is the most popular vegetable oil and is increasingly used for consumption among other vegetable oils. The cytotoxicity of the VCO-based cannabis and $ScCO_{2\ sample}$ of cannabis were determined using MTT assay. The cytotoxicity assessment of HaCaT cells was performed using a concentration range of 155−1250 ng/mL for both THC and CBD. The same range of THC concentrations was quantified in the VCO-based cannabis sample, the $ScCO_{2\ sample}$ of cannabis, and the W-$ScCO_{2\ sample}$ of cannabis. The cell viability of the tested samples is presented in Figure 4. All samples exerted nontoxic effects on HaCaT cells. The HaCaT cell viability increased significantly in some of the conditions tested for the $ScCO_2$ cannabis sample, the W-$ScCO_2$ cannabis sample, and VCO-based cannabis sample. Moreover, the viability of HaCaT cells treated with the VCO-based cannabis sample decreased slightly (but nonsignificantly) at the 1250 ng/mL THC concentration.

Photoprotective Effects of the VCO-Based Cannabis and $ScCO_2$ Cannabis Samples on UVA-Irradiated Human Skin Keratinocytes

The photoprotective effects of the VCO-based cannabis and $ScCO_{2\ sample}$ of cannabis were evaluated using MTT assay.

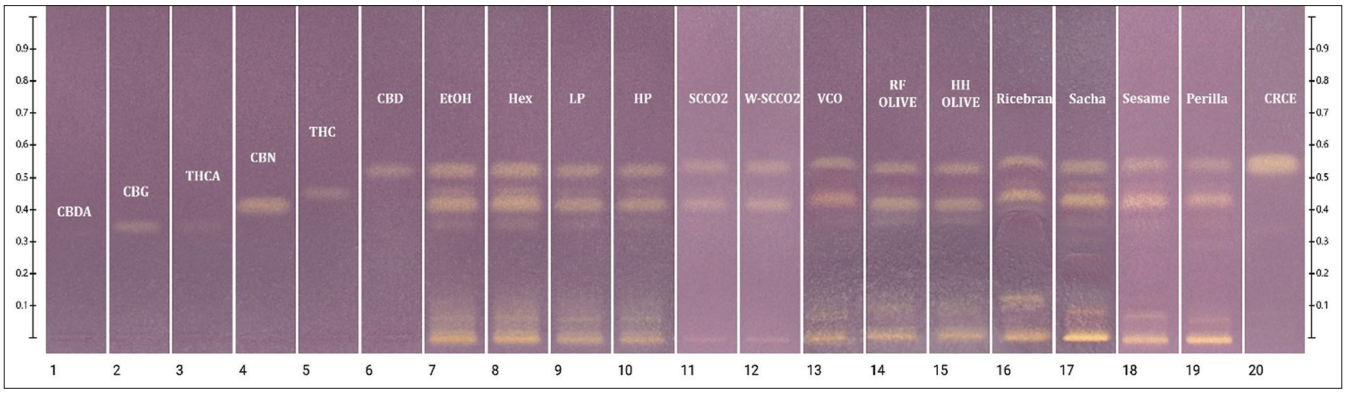

Figure 3: Thin-layer chromatography-2,2-diphenyl-1-picrylhydrazyl (DPPH) bioautography of the organic solvent extracts, $ScCO_2$ extract, and vegetable oil-based cannabis. Images taken at white light after derivatization with 0.2% DPPH methanol; track 1: Cannabidiolic acid, track 2: Cannabigerol, track 3: Tetrahydrocannabinolic acid, track 4: Cannabinol, track 5: Tetrahydrocannabinol, track 6: Cannabidiol, track 7: Ethanol, track 8: Hexane, track 9: Light petroleum ether, track 10: Heavy petroleum ether, track 11: $ScCO_2$, track 12: W-$ScCO_2$, track 13: Virgin coconut oil, track 14: Roasting and frying olive oil, track 15: High-heat olive oil, track 16: Rice bran oil, track 17: Sacha inchi oil, track 18: Sesame oil, track 19: Perilla seed oil, and track 20: Cannabidiol distillate

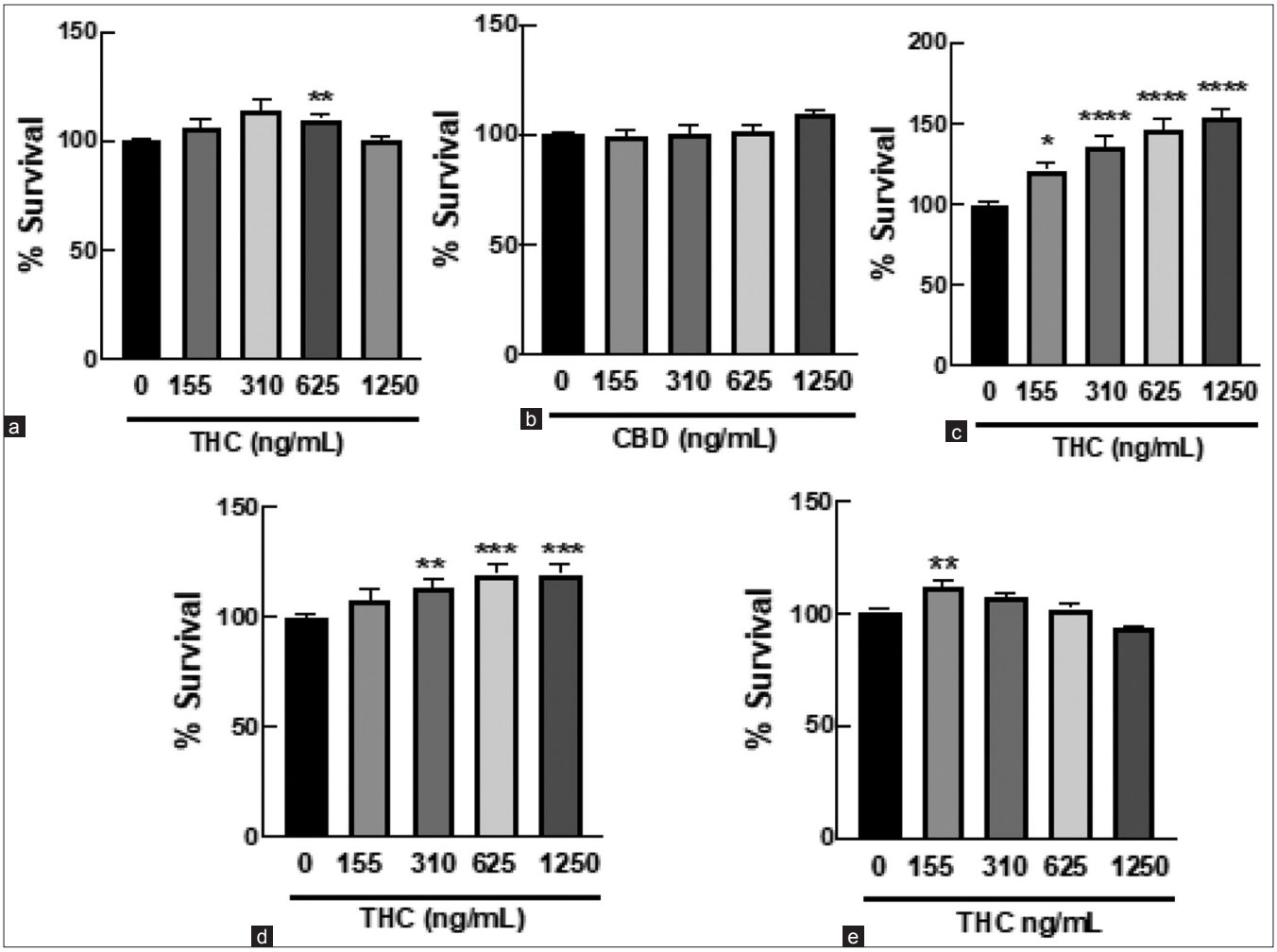

Figure 4: Cytotoxicity of 155–1250 ng/mL of (a) tetrahydrocannabinol, (b) cannabidiol, (c) $ScCO_2$ cannabis extract, (d) W-$ScCO_2$ cannabis extract, and (e) virgin coconut oil-based cannabis on human skin keratinocytes (HaCaT cells) treated for 18 h. Cell viability was assessed by MTT assay in which 0.5% DMSO was used as vehicle control (n = 3; mean ± SEM; *P < 0.05; **P < 0.005; ***P < 0.0005; ****P < 0.0001; all comparisons were made vs. the untreated control)

The viability of HaCaT cells after exposure to UVA (7 J/cm^2) is shown in Figure 5. The viability of HaCaT cells irradiated with UVA (7 J/cm^2) for 18 h decreased significantly when compared with that of untreated cells. All tested samples

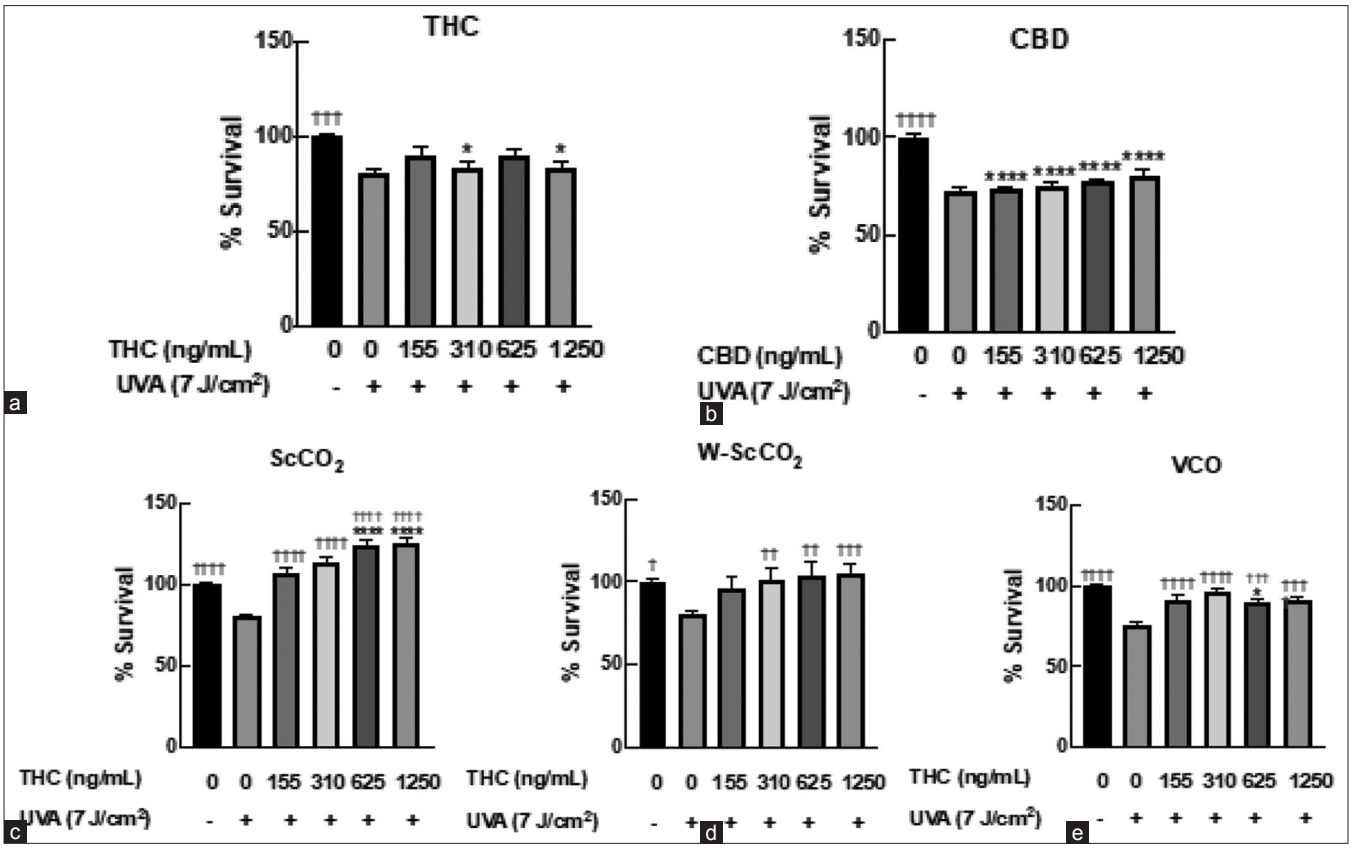

Figure 5: Photoprotective effect of 155−1250 ng/mL of (a) tetrahydrocannabinol, (b) cannabidiol, (c) $ScCO_2$ cannabis extract, (d) W-$ScCO_2$ cannabis extract, and (e) virgin coconut oil-based cannabis on UVA-irradiated HaCaT cells. The viability of HaCaT cells was assessed by MTT assay at 24 h after exposure to UVA (7 J/cm²); 0.5% DMSO was used as the vehicle control (n = 3; mean ± SEM; *P < 0.05; ****P < 0.0001; all "*" comparisons were made *vs.* the untreated control; ††P < 0.005; †††P < 0.0005; ††††P < 0.0001; all "†" comparisons were made *vs.* the UVA-irradiated control).

provided an effective photoprotection that was characterized by a significant increase in cell viability after being treated with the sample. The $ScCO_2$ and W-$ScCO_2$ cannabis samples exerted the strongest photoprotective effect on HaCaT cells; the viability of cells treated with the latter was approximately >90% and after treatment with the VCO-based cannabis as well. However, cells treated with pure THC and CBD did not show a significant difference after being irradiated with UVA. These results suggest that pure cannabinoids (such as THC and CBD) are not effective in protecting HaCaT cells against UVA radiation.

Effects of the VCO-based Cannabis and W-$ScCO_2$ Sample of Cannabis on Antioxidant Enzyme Expression in UVA-Irradiated HaCaT cells

The W-$ScCO_{2\ sample}$ of cannabis was chosen along with the VCO-based cannabis for further testing due to their higher purity than the unwinterized ($ScCO_2$) sample, as fat, chlorophyll, and wax had been removed. Oxidative stress is a major contributor to the aging of skin cells exposed to UVA.[37] Hence, the protective effects of cannabis extracts could be attributed to an enhanced capacity of the cells to detoxify oxidative insults. In this study, we observed the effects of cannabis samples on the protein expression of antioxidant enzymes (CAT and GPx-1) and of their regulators (Nrf-2 and HO-1). Western blot analysis revealed that UVA irradiation (7 J/cm²) dramatically decreased the expression of Nrf-2/HO-1, CAT, and GPx-1. Interestingly, treatment with cannabis samples significantly upregulated the protein expression levels of the enzymes [Figure 6b-d] except for GPx-1 [Figure 6e]; the latter decreased after VCO-based cannabis treatment.

DISCUSSION

Similarities of Cannabinoid Profiles and Cannabinoid Contents Observed in Vegetable Oil-Based Cannabis

Plants exhibit various pharmacological activities due to the bioactive compounds they contain. To date, HPTLC continues to attract attention and is becoming popular as an alternate method for chemical profiling, both as a solitary and complementary tool.[38,39] Chromatographic fingerprinting developed and achieved through HPTLC can be used for the verification of various phytoconstituents in plant material, thereby generating a database that can be used for future studies.[40,41] The polarity of the compound target greatly affects the choice of solvent and extraction method.[42] Furthermore, the achievement of optimal solubility of active compounds can support the success of the extraction, which

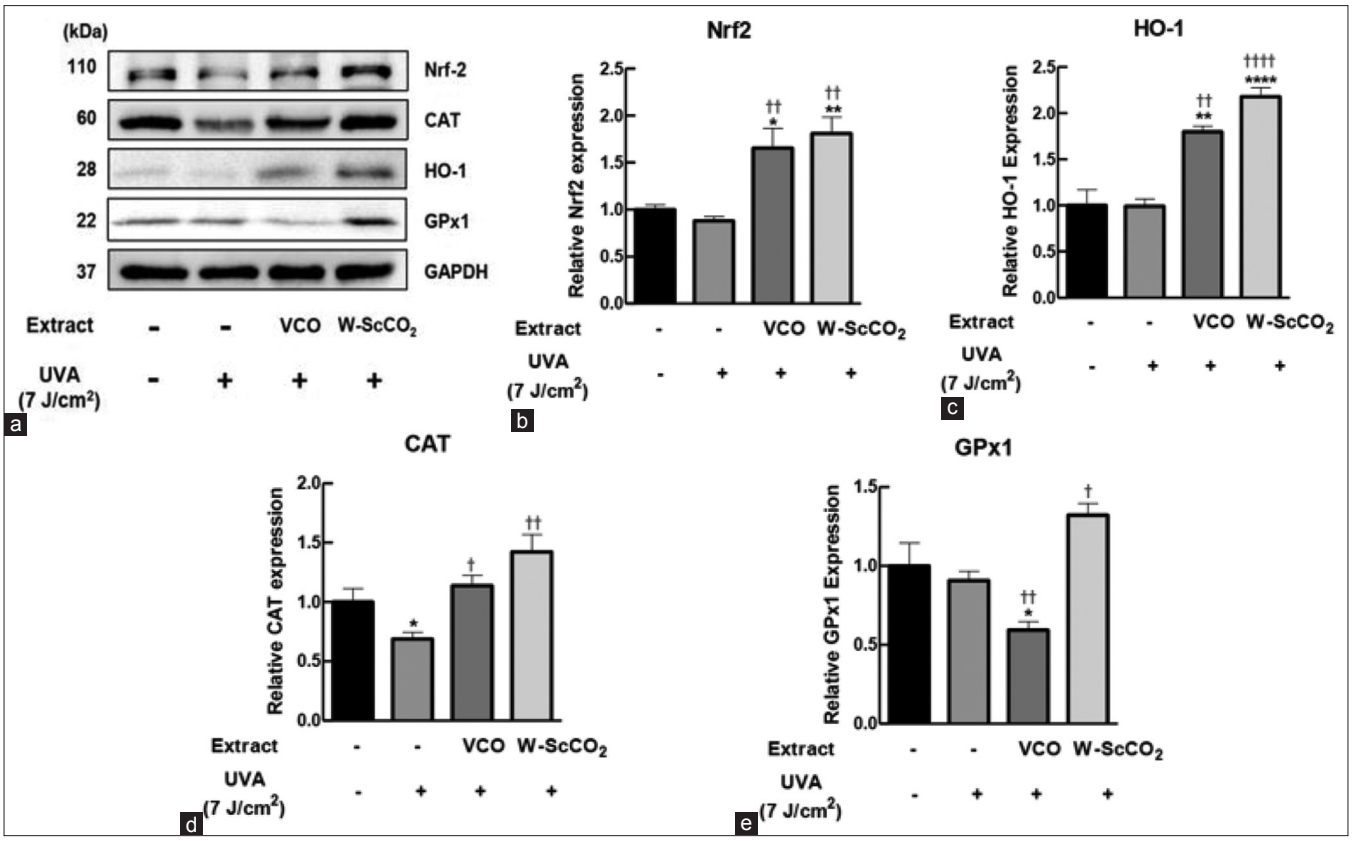

Figure 6: Antioxidant enzyme expression in UVA-irradiated (7 J/cm^2) HaCaT cells after pretreatment with virgin coconut oil-based cannabis and Winterized Supercritical CO_2 (W-ScCO_2) cannabis extract. (a) Western blot analysis. (b) Nrf-2 expression. (c) HO-1 expression. (d) CAT expression. (e) GPx-1 expression. The intensity of the bands obtained by the Western blot analysis was calculated. The expression of each protein was normalized to that of GAPDH (n = 3; mean ± SEM; $^{*}P < 0.05$; $^{**}P < 0.005$; $^{****}P < 0.0001$; all "*" comparisons were made *versus* the untreated control; $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.005$; $^{\dagger\dagger\dagger\dagger}P < 0.0001$; all "†" comparisons were made *vs.* the UVA-irradiated control).

results in the breakdown of a plant's cell structure, thereby causing an interaction between the solvent and active compound.[43] Chemically, cannabinoids are classified as nonpolar compounds, and their low solubility in water makes vegetable oils an alternative solvent option for extracting cannabinoids. The good ability of vegetable oils in dissolving several compounds has been previously reported by several studies through theoretical and experimental evidence.[44-50] This fact is also supported by the results of our study, in which HPTLC-based chemical profiling [Figure 1] revealed a similar composition between organic solvent and vegetable oil-based cannabis samples. Moreover, evidence was provided for the presence of several neutral compounds in the plant (including CBD and THC), while CBDA and THCA appeared to be absent in all tested samples. The latter is suggestive of a decarboxylation effect occurring before the extraction and leading to complete conversion of CBDA and THCA into neutral compounds.[51] Quantitatively, the effect of the vegetable oils used in this study [Figure 2a] did not differ significantly among their types in terms of their contents of THC and CBD. To the best of our knowledge, this is the first study to provide an overview of the cannabinoid profile of vegetable oil-based cannabis, which can be used for the quality control of vegetable oil-based cannabis products. Our results suggest that any vegetable oil could be used for *C. sativa* extraction; however, one should consider the composition of the base oils used as they differ in terms of their fatty acid content and odor because the vegetable oil would also be consumed together with the cannabis extract.

The Applied Extraction Method Significantly Affects Cannabinoid Content

The effects of the tested extraction methods on the THC and CBD content of the samples were compared between the cold maceration (conventional/control method) and H and F method (modified method). The results of the independent *t*-test demonstrated that the contents of THC and CBD were significantly different between the two methods [Figure 2b], with the H and F method resulting in significantly higher THC and CBD contents in all samples. The THC content in the H&F method did not differ significantly among the studied samples, except for the VCO-based cannabis when compared with the RBO-based cannabis. On the other hand, the CBD content demonstrated a significant difference ($P < 0.05$) among the studied H&F samples, except for the VCO-based cannabis when compared with the RBO-based cannabis. The SIO-based cannabis had the highest content of THC, while the VCO-based cannabis had the highest content of CBD.

The optimization of the extraction condition is a critical stage that must be thoroughly considered, and temperature is one of the parameters affecting the extraction process. In this study, the extraction method affected the quality of the

extraction, and the latter was demonstrated by a significant difference in the aforementioned compounds' content. The H&F extraction method resulted in higher THC and CBD contents than the control method. This could because the heating and frying applied on the material led to a decrease in the viscosity of the oils owing to high temperature.[52] This method can accelerate the penetration of vegetable oils into plant cells; vegetable oils with high viscosity certainly take longer to penetrate cells than those with low viscosity. Heating might also soften the plant tissues, thereby maximizing the extraction process.[53] However, an excessive increase in temperature would degrade thermolabile compounds and the stability of oils. Overheating accelerates the oxidation process, which is harmful to human health if the product is consumed. Therefore, the temperature at which the extraction process can be performed must be carefully selected. The results of this study suggest that pre- and post-extraction assessments of oil quality (such as the determination of the peroxide and iodine values, among others) are needed to monitor the changes in quality as well as safety. Notably, the increase in THC or CBD contents in H and F samples did not occur due to the conversion of acid form to neutral form because the acid forms were not detected in the samples before the extraction process, as shown in the HPLC chromatogram [Supplementary Figure 1] compared with the cannabinoid standard chromatogram that showed that the RT of cannabinoid acids peak at 5.559 (CBDA) and 14.810 (THCA), which was undetectable in the VCO-based cannabis sample before the extraction, and the amount reported in the study also includes the total amount of the acid and neutral forms.

Antioxidant Properties of *C. sativa* L.

Free radical scavenging through the use of DPPH is one of the most acceptable mechanisms for the screening and assessment of the activity of antioxidants contained in plant extracts. It is also commonly used due to the relatively short time required for the performance of the actual experiment, and it is based on the decoloration of the violet DPPH solution after the addition of the sample.[54] Our results revealed that vegetable oil-based cannabis had antioxidant activity [Table 1]. A previous study by Tan *et al.*[53] has shown that cannabinoids (such as CBN, CBD, CBG, CBDA, THCA, and THC) have antioxidant properties and that they can scavenge free radicals, induce metal ion reductions, and protect against oxidation. Based on the available literature, it is assumed that THC acts like a typical phenolic compound in removing one electron and one proton from the molecule of a cannabinoid, thereby generating a resonance-stabilized neutral radical. To the best of our knowledge, there is no report regarding the mechanism of electrochemical oxidation for other cannabinoids. Nevertheless, we believe that because cannabinoids share at least one phenolic group, it is also likely that they share similar antioxidant behavior.[55-58] The unidentified cannabinoids and the noncannabinoids contained in the sample could also contribute to the scavenging of DPPH-induced free radicals. Moreover, the presence of vegetable oil in the sample that cannot be vaporized might also contribute to the scavenging of free radicals through compounds such as tocopherol (Vitamin E) and polyphenols.[59-71] Thus, it is worth noting that not only the cannabinoids that are soluble in oil but also the oil composition itself can affect the overall antioxidant activity of the tested samples. This is also supported by the findings of our TLC–DPPH screening, which demonstrated that major cannabinoids (such as THC, CBD, and CBN) as well as unidentified compounds exerted antioxidant activities against DPPH-induced free radicals. Interestingly, RBO-based cannabis exhibited the highest IC_{50} value (3.345 ± 0.120 mg/mL), probably because of its high content of γ-oryzanol (a well-known potent antioxidant).[72]

Photoprotective Effects of the VCO-Based Cannabis and W-$ScCO_2$ Cannabis Samples on UVA-Irradiated HaCaT cells

UV radiation is one of the physical factors that can potentially and directly damage our skin cells. Excessive exposure to UV radiation can lead to skin inflammation, an imbalance of the intracellular redox status of skin cells, inhibition of skin cell proliferation, and even cell death.[37] To the best of our knowledge, this is the first study focusing on the photoprotective effects of VCO-based cannabis and W-$ScCO_2$ cannabis sample on UVA-irradiated HaCaT cells. The results of this study showed that the photoprotective effect of both samples increased HaCaT cell survival. These results are consistent with our findings demonstrating that the major cannabinoids (such as CBN, THC, and CBD) contained in cannabis have antioxidant properties. Generally, the mode of action of natural antioxidants derived from botanical compounds as skin protectors against UV radiation involves the response of the immune system, enhancement of the cells' antioxidant capacity, stimulation of anti-inflammatory cascades, activation of intracellular detoxification mechanisms, and induction of a wide gene expression alteration.[73] The photoprotective effect provided by THC and CBD was not significant in our experiments. Thus, it can be assumed that the pharmacological effect of the extract may be the result of the combination of multicompounds (not only THC and CBD). This hypothesis is supported by the antioxidant evaluation demonstrating that the IC_{50} value of the $ScCO_2$ extract was higher than that of other samples, as well as by the photoprotective effect assay revealing a higher effectiveness of the same extract in protecting the HaCaT cells from the UVA radiation compared to THC or CBD alone. Polyphenol compounds such as flavonoids and phenols isolated from cannabis are also known to possess antioxidant properties.[74]

Upregulation of Antioxidant Enzymes in UVA-Irradiated HaCaT cells after Pretreatment with the VCO-based Cannabis and W-$ScCO_2$ Cannabis Samples

In the antioxidant defense system of the cell, the cellular redox homeostasis is delicately maintained by the endogenous antioxidant system.[75] An imbalance of ROS production and ROS depletion can result in the development of oxidative stress; this phenomenon could be initiated by overexposure to UVA radiation.[76] If this imbalance persists, the endogenous antioxidant defenses collapse [Figure 6] by the downregulation of the protein expression of critical antioxidant enzymes. This study revealed that the antioxidant properties of the VCO-based cannabis and of the W-ScCO2 sample of cannabis can induce the protein expression of major antioxidant enzymes

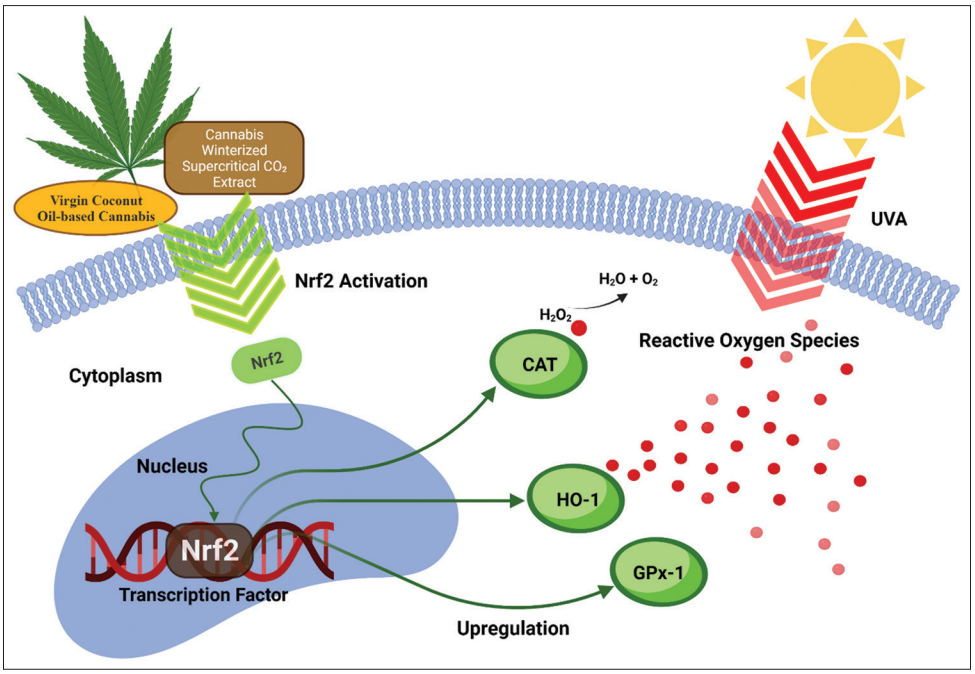

Figure 7: Upregulation of antioxidant enzymes (CAT and GPx-1) stimulated by the virgin coconut oil-based cannabis or winterized supercritical CO_2 cannabis extract through the activation of the Nrf2/HO-1 pathway in UVA-irradiated HaCaT cells (*Created with BioRender.com*)

through upregulation of the Nrf-2/HO-1 pathway [Figure 7]. More specifically, Nrf-2 expression can be stimulated by active constituents contained in the samples, with Nrf-2 acting as a transcription factor for the systemic antioxidant defense system. In this respect, it can upregulate the expression of cytoprotective genes, thereby affecting the expression of enzymes involved in the cellular antioxidant response (including HO-1, GPx-1, and CAT). Through these molecular mechanisms, both of the aforementioned samples can enhance the capacity of the keratinocytes to detoxify UVA-induced toxic effects and eventually delay/prevent the aging process of the skin cells. Hence, both the VCO-based cannabis and W-$ScCO_2$ cannabis extract would be promising candidates for use in cosmeceutical products with anti-aging applications.

CONCLUSIONS

Chromatographic fingerprinting showed that the vegetable oil-based samples of cannabis, including those extracted with VCO, olive oil, PSO, SIO, RBO, or SSO, had a similar cannabinoid profile to organic solvent samples, which also confirmed the good quality of extraction using vegetable oils. The H and F-assisted extraction also significantly increased CBD and THC contents. CBG, CBN, THC, CBD, and other unidentified compounds contained in the samples exhibited remarkable antioxidant activities. Of all the vegetable oil-based cannabis samples, the RBO-based sample demonstrated the strongest antioxidant activity. Moreover, the VCO-based cannabis sample was chosen together with $ScCO_2$ and W-$ScCO_{2\ sample}$s for the process of extraction, and they showed their ability to protect UVA-irradiated HaCaT cells. Finally, Western blotting showed that the pretreatment of HaCaT cells with some of the aforementioned samples promoted Nrf-2/HO-1 signaling, leading to an upregulation of antioxidant enzymes (such as CAT and GPx-1); therefore, the samples could increase the capacity of keratinocytes to detoxify UVA-induced oxidative insults. This study presents the cannabinoid profile of vegetable oil-based cannabis, which can be used for the purpose of quality control during the development of vegetable oil-based cannabis products. In addition, it confirms vegetable oils as promising alternative solvents and validates their other advantages, such as being beneficial to human health, affordable, and eco-friendly. Vegetables oils can be employed in the cannabis-based product development industry or even have potential cosmeceutical applications.

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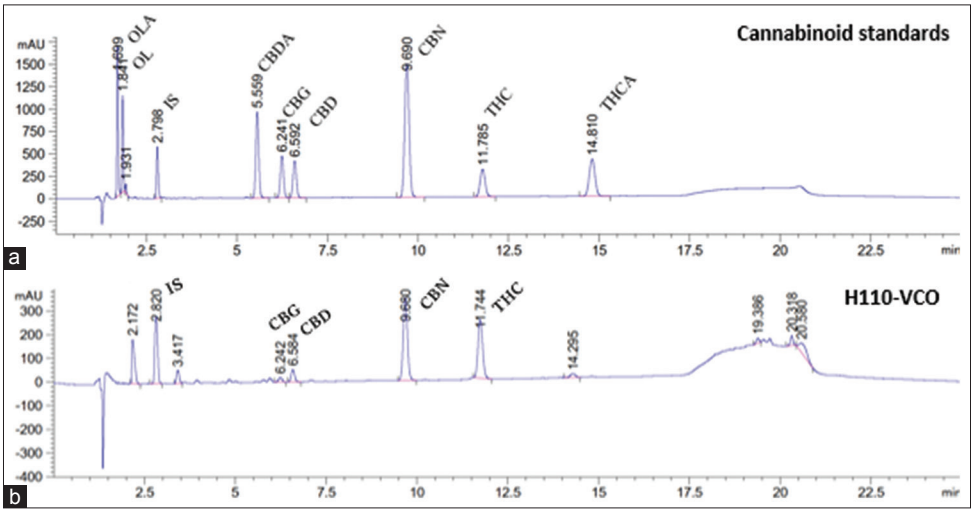
SUPPLYMENTARY FIGURE



Supplementary Figure 1: (a and b) High-performance liquid chromatography chromatograms of the cannabis sample in virgin coconut oil (VCO). The sample was preheated to 110°C for 60 min before being extracted by maceration in VCO and its cannabinoid profile was compared against cannabinoid standards