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# Apoptosis Induction on Human Cancer Cells of *Cannabis sativa* L. Cultivar Tanao Sri Kan Dang RD1 Extract

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**ABSTRACT** *Cannabis sativa* L. has been reported to be active against some kinds of malignant tumor cells. However, the mechanisms of action of the Thai cannabis, *Cannabis sativa* L. cultivar Tanao Sri Kan Dang RD1, have not been revealed. This study aimed to investigate the anti-proliferative and apoptosis-inducing properties of Tanao Sri Kan Dang RD1 extract (TRD1), cannabidiol (CBD) and Δ9-tetra-hydrocannabinol (THC). The crude extract, TRD1 was prepared from the cannabis female inflorescences using supercritical fluid extraction. The content of THC and CBD of the extracts was analyzed using High-performance liquid chromatography. The anti-proliferative properties were investigated on several cancer cell lines. The results showed that TRD1, CBD and THC posed cytotoxicity against MCF-7, Hep G2, PANC-1 and A-549 cell lines. MCF-7 cells were the most susceptible to the three compounds investigated ($IC_{50}$ = 2.81, 2.69 and 4.51 μg/mL, respectively) and chosen to investigate apoptosis induction. TRD1 induced apoptosis dose-dependently (2.5-10.0 μg/mL) in the same manner as CBD did (2.0-8.0 μg/mL), whereas THC induced apoptosis to the same extent as necrosis at 10.0 μg/mL. CBD and THC also moderated caspase-3 protein expression and induced chromatin condensation in MCF-7 cells. In conclusion, TRD1, CBD and THC exhibited anti-proliferative and apoptosis-inducing properties suggesting that they might be further investigated for their anti-cancer drug potential. In addition, in vivo pharmacological and toxicological studies should be conducted to ensure efficacy and safety for further research as medicinal product in the future.

**Keywords**: *Cannabis sativa* L. extract, Tanao Sri Kan Dang RD1, Apoptosis, Cannabidiol, Δ9-tetra-hydrocannabinol

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## Introduction

Cannabis, marijuana or *Cannabis sativa* L., is a plant that is legally licensed for medical or recreational use in many countries. Currently, the Thai government allows the legal use of cannabis for medical purposes. Thailand has been reported to be one of the habitats of *C. sativa* descendants which have the characteristics of narrow leaflets, slender and tall habit and late maturation.[1] Some cultivars of this plant have been found to have specific characteristics and intensively studied for medical purposes which are Hang Kra Rog Phu Phan ST1, Hang Suea Sakonnakhon TT1, Tanao Sri Kan Khaw WA1 and Tanao Sri Kan Dang RD1.[2] Tanao Sri Kan Dang RD1 is reported as a cannabidiol (CBD)-enriched cultivar, whereas, others presented varying amounts of THC.[2] Cannabinoids are found to be the active compounds in this plant; among these, the most common compounds are THC and CBD. The proportion of THC/CBD is also used as a criterion for the identification of cannabis strains or cultivars.[3] Besides, there have been reported other cannabinoids in the lines of phytocannabinoid synthesis such as cannabigerol (CBG), cannabichromene (CBC), Δ9-tetrahydrocannabivarin (D9-THCV), cannabicyclol (CBL), cannabinol (CBN) as final products by decarboxylation reaction. The precursors most presented in raw plants are in acid forms including f D9-THCA, CBDA and CBCA.[4] Moreover, 400 other phytochemicals have been reported to be presented including flavonoids, terpenoids, alkaloids, steroids, alkanes, fatty acids and sugars.[4,5] The pharmacological activities of cannabis were reported to focus on THC and CBD. THC and its derivatives were reported as analgesic, appetite, nausea reduction induced by chemotherapy and brain cells protection from oxidative stress. CBD, on the other hand, showed the effect of relieving epilepsy and reducing anxiety. THC, CBD and cannabinoid derivatives such as cannabichromene (CBC) and cannabigerol (CBG) or flavonoids such as apigenin, chrysoeriol, kaempferol, luteolin, quercetin, vitexin and cannflavins were found to be effective against many types of cancer cells In vivo.[5,6] CBD has also been found to be active against cancer cell lines in vitro such as breast cancer cells (MCF-7, MDA-MB-231), brain cancer cells (U87-MG, U373), lung cancer cells (NSCLC, A549), colon cancer cells (HT29), pancreatic cancer cells (PC-3, DU-145, 22RV1, and LNCaP), prostate cancer cells (PC-3, DU-145, 22RV1, LNCaP) and blood cancer cells (leukaemia/lymphoma, multiple myeloma) by inhibiting cell proliferation and angiogenesis.[7] Evasion of apoptosis is an important hallmark of cancer and the induction of apoptosis is consequently recognized as an important property of any potential therapeutic.[8,9] Therefore, research continues to focus on the elucidation and analysis of signalling pathways that control apoptosis. There are two main pathways of apoptosis; extrinsic and intrinsic pathways. Both pathways activate the executioners through caspase 3, 6 and 7. The execution pathway results in characteristic cytomorphological features including cell shrinkage, chromatin condensation, the formation of cytoplasmic blebs and apoptotic bodies and finally phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophages.[10] However, the other dichotomous mechanism of cell death is necrosis which has been characterized as accidental cell death.

The causes are chemicals, external injury or trauma of cell membrane resulting in losing the membrane integrity and releasing inflammatory cellular contents. Necrosis normally causes acute toxicity.[11]

This research, therefore, aimed to investigate the cytotoxicity of Thai cannabis strain (*Cannabis sativa* L. cultivar, Tanao Sri Kan Dang RD1) crude extract, CBD and THC in various cancer cell lines and the ability to induce apoptosis. The results of this study could be supportive information for developing Thai cannabis as an anti-cancer drug or combined chemotherapy.

## Materials and Methods

### Cell culture

Adenocarcinoma human alveolar basal epithelial cells (A-549), human Caucasian hepatocyte carcinoma cells (Hep G2), human pancreatic cancer cell line (PANC-1), and human breast adenocarcinoma cells (MCF-7) were obtained from Cell lines service GmbH (Eppelheim, Germany). A-549 cells were grown in Dulbecco's modified eagle medium (DMEM) (Gibco, NY, USA), whereas, Hep G2, PANC-1 and MCF-7 were grown in Eagle's minimum essential medium (EMEM) (Gibco, NY, USA). Both cell culture media were supplemented with 2 mM L-glutamine, 0.1 mM non-essential amino acid, 1 mM sodium pyruvate and 10% fetal bovine serum at 37°C in a 5% $CO_2$ incubator.

### Standards

Cannabidiol (CBD) (> 98% HPLC), Δ9-tetrahydrocannabinol (THC) (> 98% HPLC), cannabidiolic acid (CBDA) (> 98.5% HPLC) and tetrahydrocannabinolic acid (THCA) were form Merck KGaA (Darmstadt, Germany). Doxorubicin (> 98% HPLC grade) was purchased from Sigma-Aldrich (Buchs, Switzerland).

### Plant materials

The plant material was obtained from *Cannabis sativa* L. cultivars Tanao Sri Kan Dang RD1 (TRD1) and certified by Department of Agriculture, Ministry of Agriculture and Cooperatives (certificate no. 1688/2564). It was cultivated in the greenhouse of Medicinal Plant Research Institute, Department of Medical Sciences which had a license to grow category-5 narcotic plants (No. 90/2564, planting place). Fresh female inflorescences were collected from 6-month-old cannabis in September 2021.

### Plant extraction and quality control

TRD1 cannabis crude extract was produced using the following procedure. The fresh inflorescences were cut into small pieces about 1 cm in length. They were dried in a hot air oven at 45-50°C for 24-48 hours. The dried plant was ground into crude powder and then extracted using supercritical fluid extraction (SFE); $CO_2$ was used as the solvent to extract bioactive components. The extraction condition was set at 250 bar and 40°C. THC and CBD in the extract were analyzed using High-performance liquid chromatography (HPLC, model LC-40D, Shimadzu, Japan); with a photodiode array detector at wavelength 220 nm, column 150 × 3.0 mm C18 Infinity Lab Poroshell packed with 2.7 μm particles. The mobile phases were (A) 0.085% $H_3PO_4$ in water and (B) 0.085% $H_3PO_4$ in acetonitrile, using isocratic 30% (A): 70% (B) applied from Thongchin et al., 2021.[12] The sample was prepared by weighing 20 mg of the extract and then adding methanol:chloroform (9:1) 10 mL in a volumetric flask. The portion of 1 mL solution was adjusted to 10 mL with methanol in a volumetric flask and filtered with a 0.45 μm nylon syringe filter

before analysing by HPLC. The standards of THC, THCA, CBD and CBDA were prepared and diluted in methanol at concentrations between 3.125-100 μg/mL. The crude extract and the quality control data were conducted at the Herbal Quality Assurance Center, Medicinal Plant Research Institute, Department of Medical Sciences.

**MTT Assay**

A total of 10 mg of TRD1, THC and CBD was dissolved in DMSO to provide 10 mg/mL of stock solutions. Then the solutions were diluted to 1,000 μg/mL with the culture medium as a working solution. The cells were seeded at $5 \times 10^3$ cells in 100 μL medium per well of 96-well plates and left in the incubator for 24 hours. The medium was removed and the cells were treated with eight different concentrations of each compound in triplicate and then incubated for 48 hours. The medium was replaced with 200 μL MTT reagent and then continued to incubate for 4 hours. The MTT solution was discarded and 200 μL DMSO was added to each well to dissolve the purple formazan product. The absorbance of the formazan product of viable cells was read using the microplate reader at 570 nm. The background absorbance was reduced by the blank and %viability was calculated compared to the control.

**Quantification of Apoptosis**

A total of 10 mg of TRD1, THC and CBD was dissolved in DMSO to provide 10 mg/mL of stock solutions. Then the solutions were diluted to 1 mg/mL with the culture medium as a working solution. MCF-7 cells were seeded at $2 \times 10^5$ cells in 3 mL in 6-well plates and treated with CBD, THC and TRD1 at different concentrations for 24 hours. The cells were trypsinised and washed with ice-cold phosphate-buffered saline (PBS), and then $1 \times 10^6$ cells/mL were resuspended in 100 μL 1X annexin-binding buffer. Total 5 μL Alexa Fluor 488 annexin V and 1 μL 100 μg/mL propidium iodide (PI) working solution were added. The cells were gently mixed and incubated in the dark for 15 min at room temperature. Finally, 400 μL of binding buffer was then added to each tube and the apoptotic cells quantified using Attune™ NxT, Acoustic Focusing Cytometer (Thermo Fisher Scientific, Singapore) within 1 hour of harvesting. Cells that stained positive for FITC annexin V and negative for PI were undergoing early apoptosis. Cells stained positive for both FITC annexin V and PI were in the late apoptosis. Cells were positive for PI but negative for FITC annexin V were undergoing necrosis or were already dead. Whereas the cells that stained negative for both FITC annexin V and PI were alive.

**Caspase-3 Expression**

A total of 10 mg of THC and CBD were dissolved in DMSO to provide 10 mg/mL of stock solutions. Then the solutions were diluted to 1 mg/mL with the culture medium as a working solution. Active caspase-3 staining was performed following the manufacturer's instructions (PE active caspase-3 apoptosis kit, BD biosciences, San Diego, CA, USA). Total $5 \times 10^5$ cells of MCF-7 were treated with different concentrations of the compounds for 24 hours. The cells were harvested and washed with ice-cold PBS. The washed cells were resuspended in 0.5 mL BD Cytofix/Cytoperm™ solution and incubated on ice

for 20 min. After that the buffer was removed, the cells were washed twice with 0.5 mL BD Perm/Wash™ buffer at room temperature. Then, the cells were resuspended with 100 µL BD Perm/wash™ buffer plus 20 µL antibody and incubated for 30 min at room temperature. Finally, the cells were washed with 1 mL washing buffer and resuspended in 0.50 mL washing buffer. The cells were maintained at 4°C until analyzed by a flow cytometer.

**Nuclear morphological detection using Hoechst 33342 staining**

The nuclear morphological changes of chromatin condensation induced by CBD and THC were examined using Hoechst 33342 staining (Sigma-Aldrich, Buchs, Switzerland). The protocol was followed according to the manufacturer's instructions. Briefly, the cells were treated with different concentrations of CBD and THC in 6-well plates for 24 hours. The 10 mg/mL Hoechst stock solution was prepared and diluted with PBS (1:2,000). The medium was removed from each well and then 500 µL of the dye working solution was added. The plate was then incubated at 37°C for 10 min. The cells werethen washed with 1 mL PBS 3 times. The cells were viewed under the fluorescence microscope (Nikon Eclipse Ti2-E, Tokyo, Japan).

**Statistical Analysis**

The inhibitory concentration ($IC_{50}$) was calculated using nonlinear regression analysis (curve fit) (GraphPad Software, La Jolla, CA, United States) and expressed as mean±SD (n = 3). The total percentage of the cell population in apoptosis and caspase-3 assays was expressed as a mean of duplicated experiments.

## Results

A total of 100 g of the crude drug yielded 7.07 g of the crude extract following extraction. From the result of HPLC analysis, it was found that TRD1 contained THC, THCA, CBD, and CBDA compounds as 1.51±0.01, 3.16±0.01, 4.25±0.03, and 24.67±0.16 %w/w, respectively. The chromatogram of TRD1 is shown in Figure 1. Therefore, this extract provided the CBDA enriched extract.

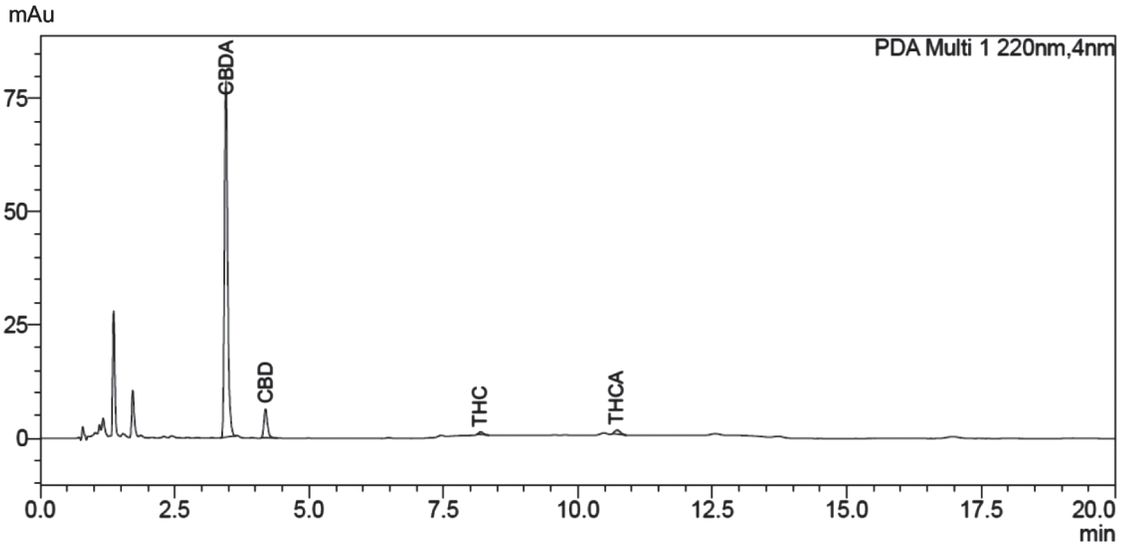


**Figure 1** Chromatogram of TRD1 extract (0.200 mg/mL), detected at 220 nm.

The anti-proliferation activity evaluated using MTT assay gave results that TRD1 possessed cytotoxicity against MCF-7, HepG2, PANC-1 and A-549 cell lines with $IC_{50}$ of 2.81±0.85, 7.65±0.49, 23.40±0.88, and 26.90±2.27 μg/mL, respectively. The two cannabinoids, CBD and THC also showed cytotoxicity consistent with TRD1 extract. CBD posed potent cytotoxicity against MCF-7 and HepG2 with $IC_{50}$ of 2.69±0.20 and 2.93±0.30 μg/mL, respectively, whereas, THC were 4.51±0.19 and 5.73±0.40 μg/mL, respectively. MCF-7 was susceptible and gave the lowest $IC_{50}$ to three compounds. The $IC_{50}$ of TRD1 was not significantly different to CBD ($p$ = 0.9637) but significantly different to THC ($p$ = 0.0160). The $IC_{50}$ of TRD1, CBD and THC to A-549, Hep G2, MCF-7 and PANC-1 cancer cell lines are shown in Table 1.

**Table 1** The 50% inhibitory concentration ($IC_{50}$) of TRD1, THC, CBD, and doxorubicin against cancer cell lines; A-549, HepG2, MCF-7, and PANC-1. The data was expressed as the mean±SD, n = 3.

| Cell lines | $IC_{50}$ (μg/mL) | | | |
|---|---|---|---|---|
| | **TRD1** | **CBD** | **THC** | **Doxorubicin** |
| A-549 | 26.90±2.27 | 4.76±1.05 | 8.03±1.02 | 0.18±0.04 |
| Hep G2 | 7.65±0.49 | 2.93±0.30 | 5.73±0.40 | 0.15±0.03 |
| MCF-7* | 2.81±0.85[(a)] | 2.69±0.20[(b)] | 4.51±0.19[(c)] | 0.10±0.01 |
| PANC-1 | 23.40±0.88 | 5.82±0.26 | 7.82±0.18 | 0.31±0.01 |

*Statistical analysis was carried out using One-way ANOVA analysis of variance followed by Tukey's multiple comparison test; a vs bns $p$-value = 0.9637, a vs c * $p$-value = 0.0160, b vs c * $p$-value = 0.0121

Apoptosis induction of TRD1 was investigated using flow cytometry. Apoptosis was induced in MCF-7 cells in a dose-dependent manner. The total cell populations (%) of apoptotic cells were 13.30±0.27, 4.27±0.09, 1.89±0.04 and 0.86±0.02% at 20.0, 10.0, 5.0 and 2.5 μg/mL, respectively (Figure 2). While there were 3.86±0.08 and 3.79±0.08% of the cell population in necrosis at the concentration of 20.0 and 10.0 μg/mL, respectively. The percentage of living cells was higher than 80% in all treated groups.

Pure CBD and THC were also investigated for their ability to induce apoptosis. CBD was found to induce apoptosis in a dose-dependent manner, with the cell population in apoptosis being 7.10±0.03, 2.16±0.04 and 0.96±0.00% at the concentrations of 8.0, 4.0 and 2.0 μg/mL, respectively (Figure 3). The result of necrotic cells was 3.16±0.80% at 8.0 μg/mL while using other doses, necrotic cells were relatively low, which was 0.39±0.10%. at 4.0 μg/mL (Figure 3). A total of cells (84.86±2.19%) was alive at 8.0 μg/mL, which was increased to more than 97% at 4.0 μg/mL. THC induced apoptosis as 9.05±0.26% of cells at 10.0 μg/mL, while 7.02±2.24% of the cell population was found to be dead at this dose (Figure 4). Cell viability remained above 80% in all concentrations treated (Figure 4).

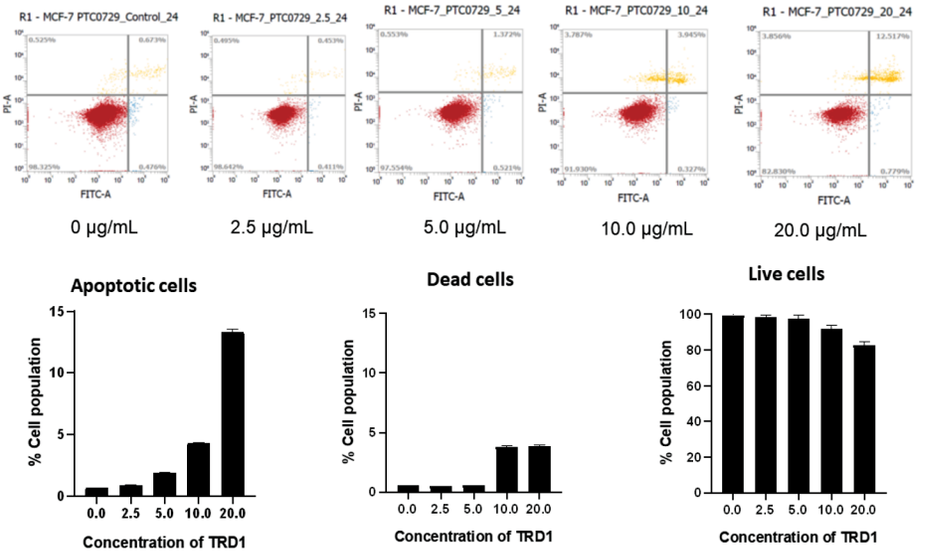


**Figure** 2 Detection of apoptosis using flow cytometry in MCF-7 cells following treatment with TRD1. Bar graphs showed the percentage of MCF-7 cells which were alive, dead or in apoptosis, following treatment with TRD1 at 2.5, 5.0, 10.0 and 20.0 µg/mL for 24 hours. The data were expressed as the mean, n = 3.

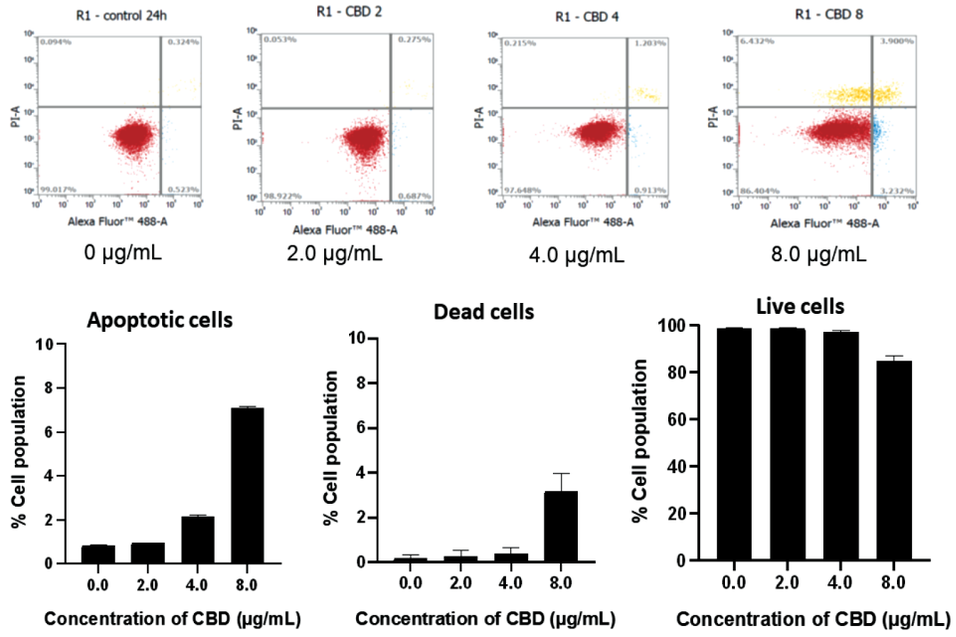


**Figure** 3 Detection of apoptosis using flow cytometry in MCF-7 cells following treatment with CBD. Bar graphs showed the percentage of MCF-7 cells which were alive, dead or in apoptosis, following treatment with CBD at 0, 2.0, 4.0 and 8.0 µg/mL for 24 hours. The data were expressed as the mean, n = 3.

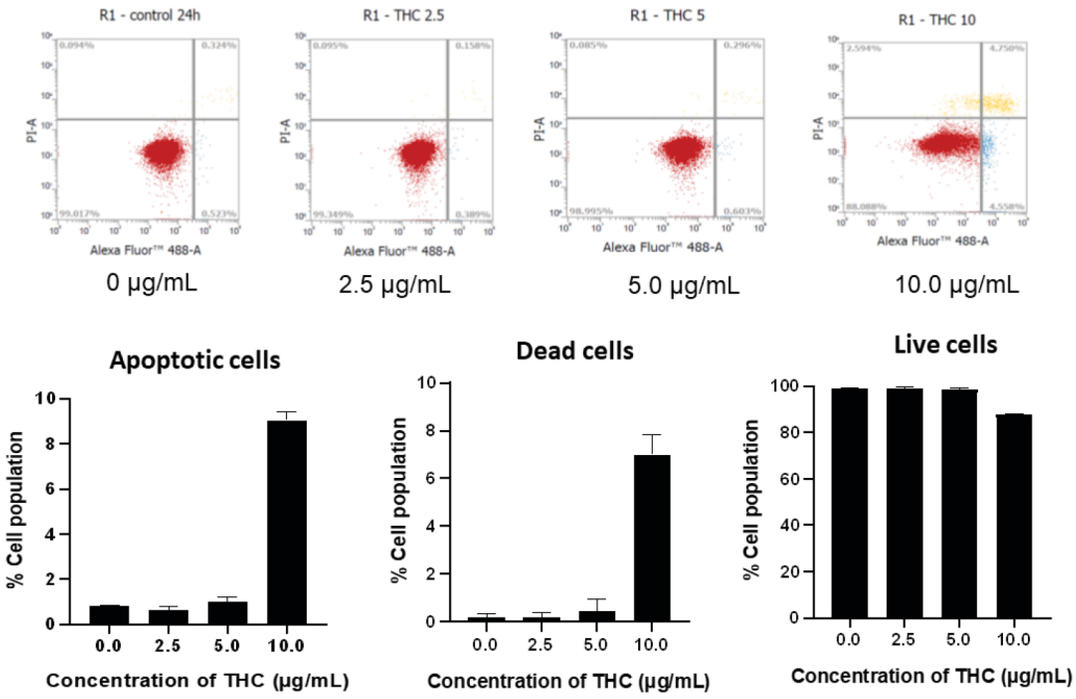


**Figure** 4 Detection of apoptosis using flow cytometry in MCF-7 cells following treatment with THC. Bar graphs showed the percentage of MCF-7 cells which were alive, dead or in apoptosis, following treatment with THC at 0, 2.5, 5.0, and 10.0 µg/mL for 24 hours. The data were expressed as the mean, n = 3.

The apoptotic executioner protein, caspase-3, was also determined using an anti-active caspase-3 antibody conjugated with PE. MCF-7 cells treated with different concentrations of CBD at 0, 2.0, 4.0 and 8.0 µg/mL for 24 hours showed a slight increase of caspase-3 expression compared to control. The total of the cell population (2.29±0.05%) was caspase-3 positive at 8.0 µg/mL. For THC, MCF-7 cells treated with different concentrations at 0, 2.5, 5.0, and 10.0 µg/mL respectively, in the same manner as CBD. It was shown that the total of the cell population was caspase-3 positive (11.29±0.05%) at 10.0 µg/mL, with lower caspase-3 expression about 0.2% at 2.5 and 5.0 µg/mL (Figure 5).

Chromatin condensation was one of the apoptotic morphological features induced following treatment. MCF-7 cells were investigated for nuclear and chromatin changes following exposure to CBD and THC (Figure 6). CBD at the concentration of 8.0 µg/mL was found to cause chromatin condensation. THC-treated cells also presented condensed chromatin at 10.0 µg/mL.

## Discussion

TRD1 was extracted from the female inflorescences of the Thai cannabis (*Cannabis sativa* L. cultivar Tanao Sri Kan Dang RD1) containing the content of CBDA at 24.67% w/w and CBD at 4.25 %w/w. CBDA has also been

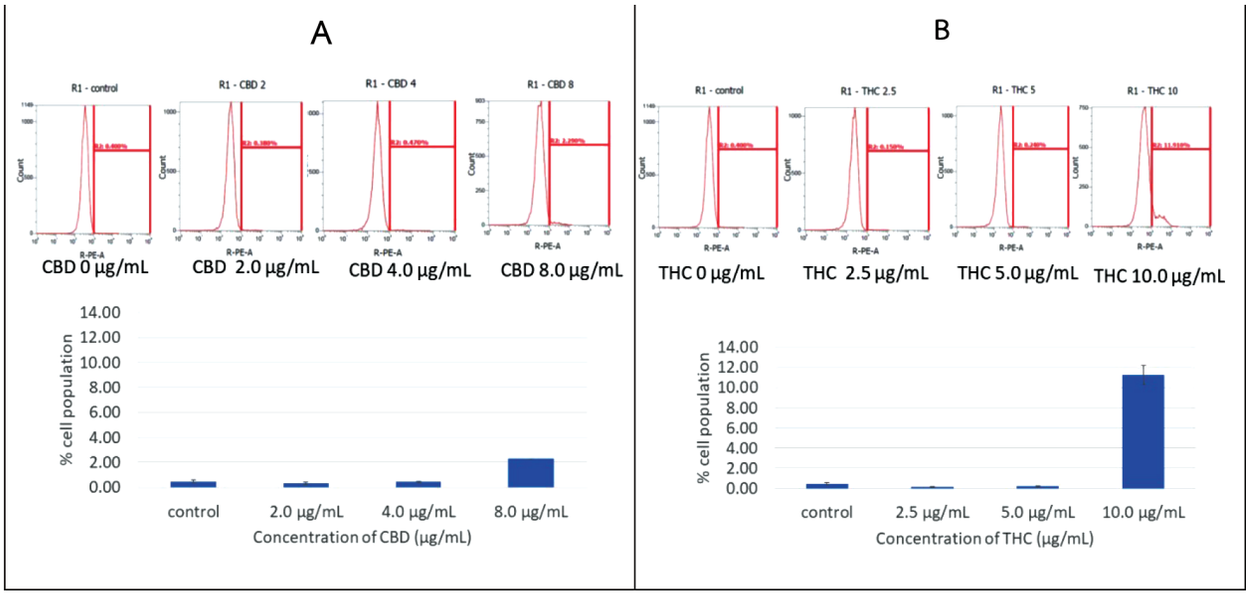


**Figure 5** Caspase-3 expression in MCF-7 cells following exposure to various concentrations of CBD (A) and THC (B) for 24 hours. Cells were stained with anti-active caspase-3 antibody and analyzed by flow cytometry. Data were the mean, n = 3.

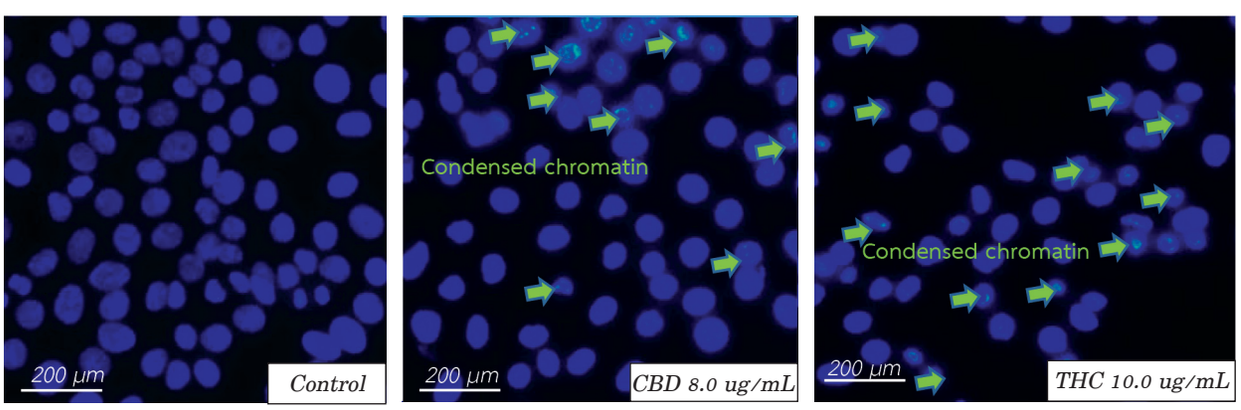


**Figure 6** MCF-7 cells treated with CBD 8.0 µg/mL and THC 10.0 µg/mL for 24 hours and then stained with Hoechst 33342 for chromatin condensation. Magnification ×20; scale bars at 200 µm. The bright green arrows indicate condensed chromatin.

reported at the highest constituents (more than 80%) of the cannabinoids extracted from inflorescences of industrial hemp using Supercritical fluid extraction (SFE)[13] that could be decarboxylated by heating or oxidizing and transformed into CBD.[14] This cannabis cultivar presented CBDA and CBD contents higher than some other cultivars such as CBD therapy (10 %w/w), Candida (CD-1) (11-20 %w/w), Juanita La Lagrimosa (7-8 %w/w).[15] The extract possessed potent cytotoxicity against breast cancer (MCF-7) and hepatic cancer cell lines (HepG2) consistent with previous studies.[16] CBDA in TRD1 extract may contribute to the inhibition and present as an active compound, which was reported to inhibit migration of highly invasive MDA-MB-231 human breast cancer cells.[17]

CBD and THC are cannabinoids, which are known to be the main constituents in this plant. The results showed that CBD was more active against the cancer cell lines than THC (Table 1). The anti-proliferative property of CBD has been previously demonstrated in several human breast cancer cell lines including MCF-10A, MDA-MB-231, MCF-7, SK-BR-3, ZR-7-1, and the PC-3 prostate cancer cells.[18] Crude extract of cannabis and its main compound CBD had also been found to be active on different cervical cancer cell lines and to pose cytotoxicity to breast cancer cell lines including EVSA-T, MDA-MB-231, MDA-MB-468 and SKBr3 cells.[19] Since CBDA and THCA were not stable, thus, CBD and THC were selected to study instead.

MCF-7 cells, the most susceptible to the compounds, therefore, were chosen to investigate apoptosis induction in this study. Cells were exposed to compounds for 24 hours to investigate the induction of apoptosis. TRD1 induced apoptosis in a dose-dependent manner. Similarly, CBD and THC also induced apoptosis dose-dependent relationship. CBD and THC had been previously reported to initiate apoptosis via activation of the cannabinoid receptor (CB). However, CBD had been reported to exert this effectindependently on CB1/CB2 receptors.[6] CBD was also reported to induce intrinsic and extrinsic apoptosis consistent with a previous study that coexisted with autophagy by inhibiting AKT and mTOR signalling.[20] THC also was reported to show anti-proliferative activity through activation of CB2 cannabinoid receptors, blocking the progression of the cell cycle and inducing apoptosis.[21] TRD1 extract showed the anti-proliferative property and induced apoptosis in the same manner as CBD did. The cytotoxic property of TRD1 might be the effect of CBDA.[17] However, apoptosis induction might be influenced not only by CBDA but also by its derivatives, THC and others.[6,20,21] THC induced apoptosis and was likely to induce necrotic dead cells at the high dose of 10.0 μg/mL. Therefore, CBD mostly caused cell death by apoptosis induction while THC at high dose caused damage to the cell membrane, necrosis induction combined with apoptosis.

Caspases play an effective role in the execution of apoptosis either through the extrinsic or intrinsic pathway.[10] In this study, caspase-3 enzyme was analyzed following treatment with CBD or THC for 24 hours. CBD and THC at high concentration of 8.0 and 10.0 μg/mL, respectively, activated caspase-3 protein. Caspase-3 protein induced by CBD was lower than the induction induced by THC. However, THC was reported to inhibit Fas-induced caspase-3 activity in A-549 cells without directly affecting the caspase-3 enzyme.[22] Apoptosis induction of CBD and THC in MCF-7cells was characterized by morphological changes. The events included condensation of chromatin and formation of apoptotic blebs within the plasma membrane, cell shrinkage due to the leakage of organelles in the cytoplasm as well as the presence of bright blue stained cells at high concentrations. The CBD-treated cells were observed to display chromatin condensation in some cells. Chromatin condensation and the formation of apoptotic blebs within the plasma membrane of apoptotic cells induced by CBD and CBD-enriched cannabis extracts had been previously reported in Cervical cancer cell lines.[13]

Thai CBDA-enriched cannabis extract, TRD1, and CBD played a major non-psychoactive constituent of cannabis, providing anti-proliferative properties in several cancer cell lines. The crude extract and CBD also induced apoptosis induction. These compounds should be considered as a potential candidate for cancer drug development.

## Conclusion

Thai cannabis extract, TRD1, CBD and THC displayed anti-proliferative properties against A-549, HepG2, PANC-1 and MCF-7 cancer cell lines. TRD1 extract at 2.5, 5.0, 10.0 and 20.0 μg/mL showed anti-proliferative property and induced apoptosis in the dose-dependent relationship as same as CBD at 2.0, 4.0, 8.0 μg/mL in MCF-7 cells. THC induced apoptosis to the same extent as necrosis at the high dose (10 μg/mL). CBD and THC moderated caspase-3 protein expression and induced chromatin condensation.

## Acknowledgement

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## References

1. McPartland JM. Cannabis systematics at the levels of family, genus, and species. Cannabis Cannabinoid Res 2018; 3(1): 203-12.
2. Saepae P. Get to know Thai marijuana and its medical purpose use. [online]. 2022; [cited 2022 Sep 20]; [10 screens]. Available from: URL: https://science.mahidol.ac.th/simple-science/2022/04/21/know-about-thai-cannabis.
3. Small E, Cronquist A. A practical and natural taxonomy for Cannabis. Taxon 1976; 25: 405-35.
4. Bonini SA, Premoli M, Tambaro S, Kumar A, Maccarinelli G, Memo M, Mastinu A. Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. J Ethnopharmacol 2018; 227: 300-15.
5. Moreau M, Ibeh U, Decosmo K, Bih N, Yasmin-Karim S, Toyang N, et al. Flavonoid derivative of cannabis demonstrates therapeutic potential in preclinical models of metastatic pancreatic cancer. Front Oncol 2019; 9: 660. (9 pages).
6. Daris B, Verboten MT, Knez Z, Ferk P. Cannabinoids in cancer treatment: therapeutic potential and legislation. Bosnian J Basic Med Sci 2019; 19(1): 14-23.
7. Zhelyazkova M, Kirilov B, Momekov G. The pharmacological basis for application of cannabidiol in cancer chemotherapy. Pharmacia 2020; 67(4): 239-52.
8. Pfeffer CM, Singh ATK. Apoptosis: a target for anticancer therapy. Int J Mol Sci 2018; 19(2): 448. (10 pages).
9. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5): 646-74.
10. Kruidering M, Evan GI. Caspase-8 in apoptosis: the beginning of "the end"? IUBMB Life 2000; 50(2): 85-90.
11. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infec Immu 2005; 73(4): 1907-16.
12. Thongchin T, Marsud S, Thiemthieprat P, Ruengkhet S, Ontong S, Banyati P, et al. Development and method validation of cannabinoid contents In cannabis sativa L. leaves by ultra high performance liquid chromatography. Bull Med Sci 2021; 63(3): 506-23.

13. Jokić S, Jerković I, Pavić V, Aladić K, Molnar M, Kovač MJ, et al. Terpenes and cannabinoids in supercritical CO2 extracts of industrial hemp inflorescences: optimization of extraction, antiradical and antibacterial activity. Pharmaceuticals 2022; 15(9): 1117. (21 pages).
14. Radapong S, Suppajariyawat P, Phadungkit. M. Pharmacological and toxicological effects of cannabis. Bull Med Sci. 2020; 62(1): 219-32.
15. Suwan W. A variety of cannabis species. [online]. 2022; [cited 2022 Jan 17]; [4 screens]. Available from: URL: https://erp.mju.ac.th/acticleDetail.aspx?qid=1065.
16. Radapong S, Suppajariyawat P, Sahad T, Harnkit N, Phankhajon K, Primprai P, et al. Investigation of the cytotoxicity of Thai cannabis extracts on various cancer cell lines. Bull Med Sci 2021; 63(3): 456-66.
17. Takeda S, Okajima S, Miyoshi H, Yoshida K, Okamoto Y, Okada T, et al. Cannabidiolic acid, a major cannabinoid in fiber-type cannabis, is an inhibitor of MDA-MB-231 breast cancer cell migration. Toxicol Lett 2012; 214(3): 314-9.
18. Alexander A, Smith PF, Rosengren RJ. Cannabinoids in the treatment of cancer. Cancer Lett 2009; 285(1): 6-12.
19. Lukhele ST, Motadi LR. Cannabidiol rather than Cannabis sativa extracts inhibit cell growth and induce apoptosis in cervical cancer cells. BMC Complement Altern Med. 2016; 16(1): 335. (16 pages).
20. Shrivastava A, Kuzontkoski PM, Groopman JE, Prasad A. Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. Mol Cancer Ther 2011; 10(7): 1161-72.
21. Caffarel MM, Sarrió D, Palacios J, Guzmán M, Sánchez C. Delta9-tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. Cancer Res 2006; 66(13): 6615-21.
22. Sarafian TA, Tashkin DP, Roth MD. Marijuana smoke and Delta(9)-tetrahydrocannabinol promote necrotic cell death but inhibit Fas-mediated apoptosis. Toxicol Appl Pharmacol 2001; 174(3): 264-72.

# ฤทธิ์เหนี่ยวนำให้เกิดการตายแบบอะพอพโทซิสในเซลล์มะเร็งของสารสกัดกัญชาพันธุ์ตะนาวศรีก้านแดง อาร์ดี วัน

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**บทคัดย่อ** กัญชาออกฤทธิ์ยับยั้งเซลล์มะเร็งหลายชนิด ยังไม่มีรายงานกลไกการออกฤทธิ์ของสารสกัดกัญชาไทยพันธุ์ตะนาวศรีก้านแดง อาร์ดี วัน (*Cannabis sativa* L. cultivar Tanao Sri Kan Dang RD1) การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ต้านเซลล์มะเร็งและการเหนี่ยวนำการตายของเซลล์แบบอะพอพโทซิสของสารสกัดกัญชาพันธุ์ตะนาวศรีก้านแดง อาร์ดี วัน (TRD1) cannabidiol (CBD) และ Δ9-tetrahydrocannabinol (THC) สารสกัดหยาบกัญชา TRD1 ถูกสกัดจากช่อดอกตัวเมียของกัญชาโดยใช้วิธีของไหลวิกฤตยิ่งยวด วิเคราะห์ปริมาณ CBD และ THC ในสารสกัดด้วยวิธีโครมาโทกราฟฟีของเหลวสมรรถนะสูง (HPLC) เมื่อทดสอบความเป็นพิษต่อเซลล์มะเร็งเพาะเลี้ยงด้วยวิธี MTT assay พบว่า TRD1, CBD และ THC ก่อให้เกิดความเป็นพิษต่อเซลล์ MCF-7, Hep G2, PANC-1 และ A-549 โดย TRD1, CBD และ THC มีฤทธิ์ต้านเซลล์มะเร็ง MCF-7 มากที่สุด มีค่า $IC_{50}$ เท่ากับ 2.81, 2.69 และ 4.51 µg/mL ตามลำดับ สารสกัด TRD1 ต้านการเจริญของเซลล์มะเร็งและเหนี่ยวนำการตายแบบอะพอพโทซิส ในลักษณะแปรผันตามความเข้มข้นของสาร (2.5-10.0 µg/mL) เช่นเดียวกับ CBD (2.0-8.0 µg/mL) ส่วน THC ที่ความเข้มข้น 100 µg/mL เหนี่ยวนำให้เกิดการตายแบบอะพอพโทซิสในระดับที่ใกล้เคียงกับเนโครซีส นอกจากนี้พบว่า CBD และ THC กระตุ้นการแสดงออกของโปรตีน caspase-3 และการรวมตัวของโครมาตินของเซลล์ MCF-7 ผลการศึกษาฤทธิ์ต้านเซลล์มะเร็งโดยการเหนี่ยวนำให้เกิดการตายแบบอะพอพโทซิสของ TRD1, CBD และ THC แสดงถึงศักยภาพในการพัฒนากัญชาพันธุ์ไทยเป็นยาต้านมะเร็งต่อไป อย่างไรก็ตามควรศึกษาฤทธิ์ทางเภสัชวิทยาและพิษวิทยาในสัตว์ทดลอง เพื่อให้ทราบประสิทธิผลและความปลอดภัยสำหรับการพัฒนาเป็นผลิตภัณฑ์ยาในอนาคต

**คำสำคัญ:** สารสกัดกัญชา, ตะนาวศรีก้านแดง อาร์ดี วัน, อะพอพโทซิส, Cannabidiol, Δ9-tetrahydrocannabinol