α-Glucosidase inhibitors from the leaves of *Cannabis sativa* and cannabinoid derivatives

Areeba Nayab[1], Nguyen Anh Khoa[1] and Preecha Phuwapraisirisan[1*]

[1] Center of Excellence in Natural Products, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
*Corresponding author, Email: preecha.p@chula.ac.th

Abstract

Cannabinoids are naturally occurring secondary metabolites that possess antidiabetic, anti-inflammatory, and anticancer compounds because of their enormous interaction efficiency with the human endocannabinoid system. The most abundant cannabinoid that is present in *Cannabis sativa* L. (commonly called cannabis) is delta-9-tetrahydrocannabinol (Δ^9-THC). Δ^9-THC has shown potential effects in alleviating the pain and nausea induced by cancer. It also acts as a major antidiabetic drug. However, the structure-activity profile of THC derivatives has not yet been demonstrated. This study focused on the isolation of cannabinoid series from the leaves of cannabis to gain insight into the structure-activity relationship (SAR) of THC derivatives. Six compounds were isolated and one THC derivative was synthesized. Their structures were characterized mainly by NMR spectroscopic data. The isolated and synthesized compounds were evaluated for inhibitory effect against α-glucosidases. Of the compounds examined, delta-9-tetrahydrocannabinol (Δ^9-THC) and cannabinol (CBN) proved to have maximum inhibitory activity, showing IC_{50} values of 268.3 and 211.8μM, respectively.

Keywords**:** *Cannabinoids, α-glucosidase, Derivatives, Cannabis, Antidiabetic, Structure-activity relationship*

1. Introduction

Diabetes mellitus is a progressive metabolic condition defined by hyperglycemia due to the lack of insulin production and insulin resistance. Mostly, it is characterized as 1 of 2 types, comprising type I and type II. The common cause of type I is the loss of beta cells, resulting in complete insulin secretion shutdown. Type II diabetes is concerned with insulin levels. Beta cells start showing dysfunction, leading to decreased levels of insulin secretion. It is the most common endocrine issue considered a significant global health challenge in the 21st century (Salim, 2005). Drug therapy, insulin injections, and lifestyle modifications are the conventional methods used to treat diabetes mellitus. To treat type II DM, α-glucosidase inhibitors can be used as a first-line medication. α-glucosidase inhibitors work by alleviating carbohydrate digestion in the small intestine. As a result, the post-prandial blood glucose level is reduced (Derosa & Maffioli, 2012). Pharmaceutical examples of α-glucosidase inhibitors include acarbose and miglitol. Abdominal bloating, hypersensitivity, and diarrhea are some of the adverse effects of acarbose and miglitol medication. However, natural remedies from medicinal plants are being considered for the medication and treatment of diabetes. Some recognized examples of α-glucosidase inhibitors from natural sources often present in herbal medicines, such as deoxy nojirimycin from *Morus alba* and kotalanol from *Salacia reticulata* (Salehi et al., 2019).

In the search for natural medicinal plants that can treat diabetes through α-glucosidase inhibition, *Cannabis sativa*, commonly known as hemp, has proven to be quite intriguing in terms of hydrocarbons that are mixed biosynthetic secondary metabolites. These hydrocarbons are called cannabinoids and around 200 naturally occurring cannabinoids are being isolated and identified. Tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) are some of the important and commonly occurring cannabinoids that have

shown maximum psychoactive effects (Coetzee, Levendal, Van de Venter, & Frost, 2007). The discovery of delta-9-tetrahydrocannabinol (Δ^9-THC) brought remarkable prosperity to the field of therapeutic medicine as it is a highly potent psychoactive drug. Cannabinoids have a special affinity towards cannabinoid receptors (CB1 and CB2) in mammals (Kovalchuk & Kovalchuk, 2020). For treating several diseases, these receptors have proven to be of prime importance.

In recent decades, a significant amount of research has been done on the therapeutic profile of cannabis. Major cannabinoids have shown potent effects against cancer activity, as studied by Velasco et al. (2007). They suggested that cannabinoids, especially THC, showed remarkable strength in lowering the growth of tumor cells due to their abundance, including glioma cells. Furthermore, Vučković, Srebro, Vujović, Vučetić, & Prostran (2018) reported that THC capsules (Marinol-TM and Cesamet-TM) proved to be extremely effective for the treatment of nausea and vomiting caused by chemotherapy. Phytocannabinoids are being used extensively for the treatment of pain. During the analgesic mechanism, cannabinoids mitigate the release of neurotransmitters from presynaptic nerve endings and activate the descending pain pathway inhibition, thus reducing neural inflammation (Vučković, Srebro, Vujović, Vučetić, & Prostran, 2018).

Campos, Moreira, Gomes, Del Bel, & Guimaraes (2012) demonstrated that another cannabinoid "cannabidiol (CBD)" also has numerous effects in treating anxiety disorders including post-traumatic stress disorder (PTSD), schizophrenia, and epilepsy. The interaction of CBD with serotonin receptors and the endocannabinoid system is thought to be the key to its anxiolytic effects. CBD has also been studied for its potential anti-inflammatory effects as well as neuroprotective properties. These characteristic pharmacological advantages make CBD suitable for treating Alzheimer's disease and Parkinson's disease. Mammalian studies of CBD have also highlighted its importance in reducing neuroinflammation and oxidative stress.

Cannabinoids show potential therapeutic effects in treating different metabolic disorders, most importantly, diabetes. Weiss et al. (2006) studied the effects of CBD on the onset of diabetes. They used non-obese diabetic mice in their studies and concluded that CBD predominantly lowers the risk of diabetes incidence as well as suppresses the effects of cell-mediated immunity and inflammation. Another related issue of diabetes is diabetes-induced oxidative stress. The potential damage of this condition is neural cell death and blood-retinal barrier. El-Remessy et al. (2006) conducted a different diabetic experiment using CBD as a therapeutic agent. They concluded that CBD showed considerable activity for reducing oxidative and nitrative stress 3 and actively protected the ganglion cells of the retina from apoptosis. CBD blocks lipid peroxidation or can form nitrotyrosine in NMDA-induced neurotoxicity in rats. As a result of this, CBD prevents the expression of proinflammatory cytokines.

Suttithumsatid, Shah, Bibi, & Panichayupakaranant (2022) recently discovered the inhibitory effect of Δ^9-THC, CBD, and standardized cannabis extract against α-glucosidase. They used molecular docking to examine the effectiveness of cannabinoids on α-glucosidase inhibitors. Among the tested compounds, Δ^9-THC and CBD showed an IC_{50} of 3.0 µg/ml and 5.5 µg/m, respectively, while the standard drug acarbose showed $1C_{50}$ of 488.6 µg/ml. According to their research, it was clear that for in vitro studies, Δ^9-THC shows the strongest binding affinity with the receptor site of the targeted protein.

In the current literature, there exists a noteworthy gap in providing the full therapeutic profile of cannabis other than Δ^9-THC and CBD. Most work has only been related to discussing the therapeutic effects of THC and CBD, resulting in a substantial lack of understanding and demonstrating the full spectrum of phytochemicals in cannabis. Figure 1 shows the compounds isolated and synthesized from cannabis leaves.

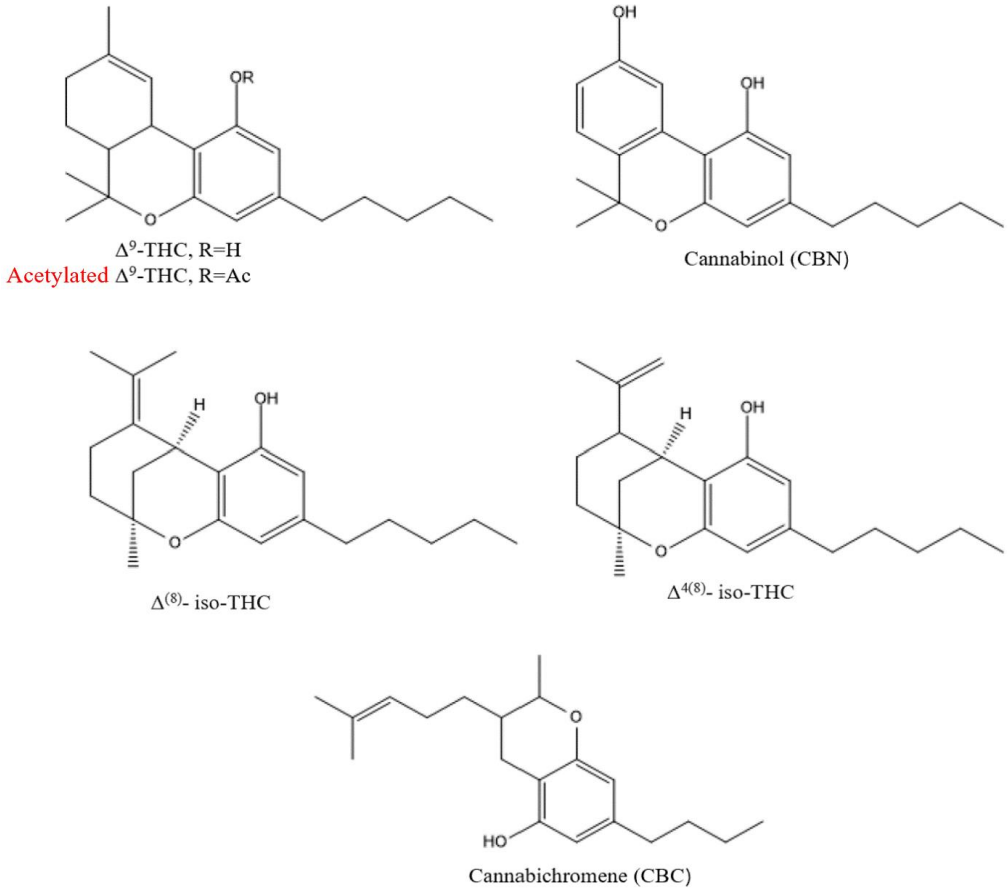

Figure SEQ Figure * ARABIC 1. Structures of isolated and synthesized compounds

2. Objectives

The main objectives of this work were:

1. To isolate and identify more compounds having comparable therapeutic effects similar to THC and CBD
2. To demonstrate the structure-activity relation of THC derivatives that hold promise for increasing the efficacy of antidiabetic effects

3. Materials and Methods

^{1}H NMR spectra were recorded by JEOL JNM-ECZ500R/S1 at 500 MHZ. Monitoring of the reaction and identification was performed by analytical thin-layer chromatography (TLC). Chromatographic plates, pre-coated with Merck silica gel 60 F_{254} (0.25 mm for thick layer), were utilized for TLC and visualized at 254nm under a UV lamp followed by spraying with 3%H_2SO_4 or 3% *p*-anisaldehyde in methanol and heating. Merck silica gel 60 (70-230 mesh) and LH-20 were used for performing column chromatography.

3.1 Plant material

The leaves of *C. sativum* were collected from a plantation area in Kanchanaburi Province, Thailand in May 2020. The specimens were stored at the Center of Excellence in Natural Products, Chulalongkorn University until used.

3.2 Extraction and isolation

The leaves (1 Kg) of *Cannabis sativa* L. were dried at 50°C in the oven for 2 hours. The dried leaves were ground and soaked in methanol (2×3.5 L) overnight. To get the crude methanolic extract, the solvent was evaporated using a rotary evaporator. The methanol extract (48 g) was chromatographed on silica gel column chromatography using the ethyl acetate–hexane mixture (0:1→1:0, stepwise increasing polarity elution). This partitioning yielded 3 fractions (F1–F3) containing major cannabinoids in F1 (7g), which were confirmed by thin layer chromatography (TLC) and NMR. This fraction was subjected to suitable silica gel and Sephadex column chromatography for further purification. Orangish red fraction F1 was further subjected to silica gel column chromatography and eluted with (3:97) EtOH: *n*-hexane, which resulted in 5 subfractions (F1.1-F1.5). Sephadex LH-20 fractionated the fraction F1.4 (500 mg) eluted with (1:1) MeOH-CH_2Cl_2 to give pure cannabinol (50mg) and a subfraction F1.4(a). Subfraction F1.4 (a) 230mg was fractionated by silica gel column eluting with (40:60) CH_2Cl_2: *n*-hexane afforded two compounds, delta-9-tetrahydrocannabinol (Δ^9-THC) (150 mg) and cannabichromene (75mg). Sephadex LH-20 fractionated subfraction F1.3 (250 mg) eluted with MeOH-CH_2Cl_2 (7:3, v/v) to give a subfraction F1.3 (a), which was subjected to silica gel column and eluted with (96:2:2) *n*-hexane: toluene: DEA that afforded two structural isomers, namely Δ^8- iso -tetrahydrocannabinol (2 mg) and $\Delta^{4(8)}$- iso -tetrahydrocannabinol (3 mg), as light-yellow amorphous solids. Figure 2 illustrates a schematical diagram of the compounds isolated from the dried leaves of cannabis.

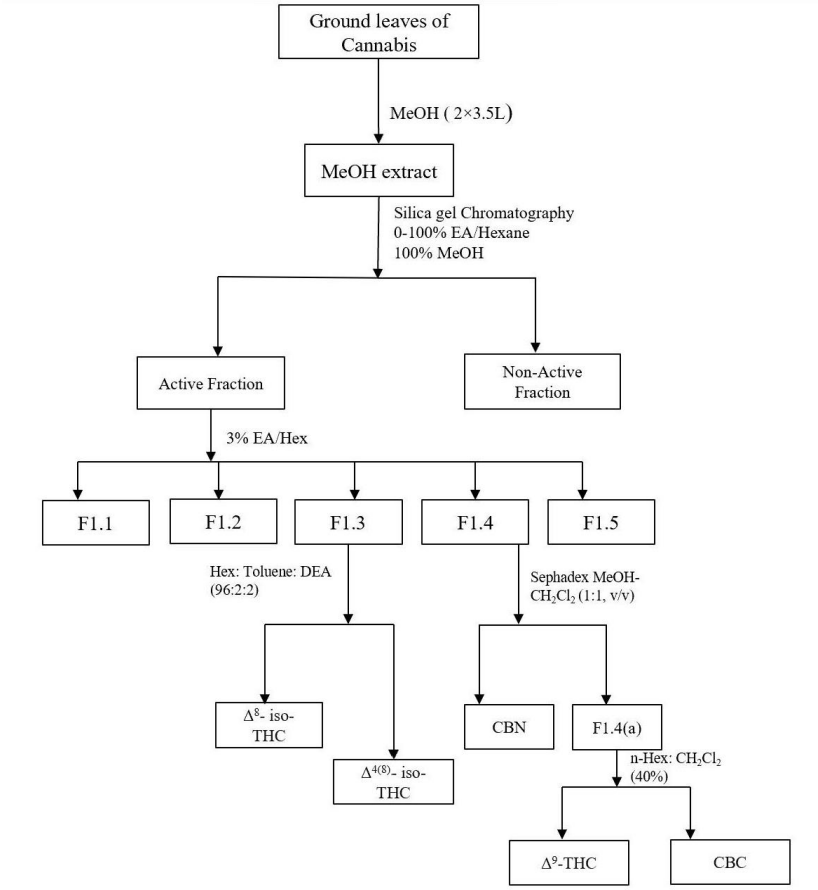

Figure 2. Schematical representation showing the isolation of cannabinoids from raw materials

3.3 Modification of tetrahydrocannabinol

At room temperature, Δ^9-THC (150 mg, 0.5 mmol, 1 equiv.) was dissolved in 1 mL dichloromethane. Dimethylallyl pyrophosphate (DMAPP) was added to the solution and stirred for 10 minutes. A few drops of acetic anhydride were added, after which the reaction mixture was stirred for 30 min. After the reaction was completed and monitored by TLC, purification of the product was performed by silica gel chromatography eluted with (5:95) EtOH: *n*-hexane. Acetylated THC (100 mg) was obtained as a yellow amorphous liquid in 80% yield. Figure 3 describes the complete acetylation reaction of THC.

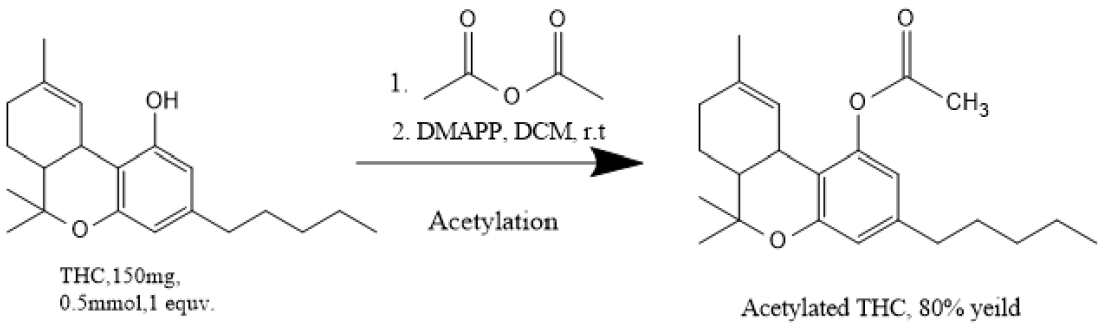

Figure 3. Acetylation of tetrahydrocannabinol

3.4 α-Glucosidase inhibitory activity

α-Glucosidase inhibition was evaluated using the colorimetric method previously described by Ramadhan & Phuwapraisirisan (2015). The enzyme solution was prepared as a source of sucrase and maltase using rat intestinal acetone powder (Sigma, St. Louis). Phosphate butter (30 µL) of 6.9 pH was added to the isolated compounds. Maltose and sucrose were added as substrates in the prepared solution of buffer in different concentrations [10 mM maltose and 100 mM of sucrose (each 20µL volume)]. Finally, an 80µL volume of glucose assay kit (SU-GLLQ2, Human, 80 µL) and a 20 µL volume of enzyme solution were added to the phosphate buffer solution. The mixture was incubated for different times depending on the substrates [10 minutes (maltose) and 40 minutes (sucrose)] at room temperature. Incubation permits the enzyme to utilize substrate molecules into glucose. The amount of resulting glucose was proportional to quinoneimine, which showed absorption at a wavelength of 520 nm on a Bio-Rad 3550 microplate reader. For calculating percentage inhibition, the following formula was used.

$$B = [(A0-A1)/A0] *100$$

Where A0 = Absorbance without sample

A1 = Absorbance with sample

4. Results and Discussion

The isolated and acetylated tetrahydrocannabinol compounds were evaluated for the inhibition of α-glucosidase based on the previously described procedure. Only cannabinol (CBN) showed maximum activity for α-glucosidase inhibition against maltase.

Tetrahydrocannabinol (THC) and cannabichromene (CBC) showed less inhibitory activity than cannabinol using the calorimetric method previously described by Ramadhan & Phuwapraisirisan (2015). Δ^{8}-iso -THC and $\Delta^{4(8)}$- iso -THC and acetylated-THC showed no inhibition, even though they are isomers of tetrahydrocannabinol. To the authors' knowledge, cannabis leaves are a mixture of cannabinoids, flavonoids and terpenoids but comprise relatively low concentrations of cannabinoids (1-2%) compared to cannabis inflorescence (Jin, Dai, Xie, & Chen, 2020). Proença et al. (2017) likewise found that cannabis leaves were a mixture of cannabinoids, flavonoids and terpenoids but comprised relatively low concentrations of cannabinoids (1-2%) when compared to cannabis inflorescence. Previously, it was suggested that polarity and the presence of phenolic moiety play a vital role in the inhibition of α-glucosidas (Nguyen, & Phuwapraisirisan, 2024). The general inclination is that more polar cannabinoids results in more potent inhibitors. The inhibition against α-glucosidase was elevated with increasing phenolic groups and vice versa. Compared to THC having an activity of 268.3 µM, CBN has two phenolic groups, making it a more favorable candidate for the inhibition of α-glucosidase. That is why CBN showed higher activity against maltase (211.8 µM) compared to THC. Cannabichromene (CBC) has one phenolic group, but the core structure has a long

aliphatic region, making the compound more hydrophobic. However, the activity of CBC (297.8 μM) is comparable to CBN and THC. Δ^8- iso -THC and $\Delta^{4(8)}$ - iso -THC are the isomers of Δ^9-THC. They also have the same number of phenolic groups, but these compounds have an open cyclic ring on one side, making them less active than other compounds. Moreover, polarity plays a vital role in the activity of the compounds. Among all the isolated compounds, Δ^8- iso -THC and $\Delta^{4(8)}$ - iso -THC were found to have the least polarity as they appeared with a maximum rf value on the TLC plate, making them a less favorable candidate for the inhibition of α-glucosidase. Figure 4 shows the TLC profiles of all the isolated compounds from cannabis leaves.

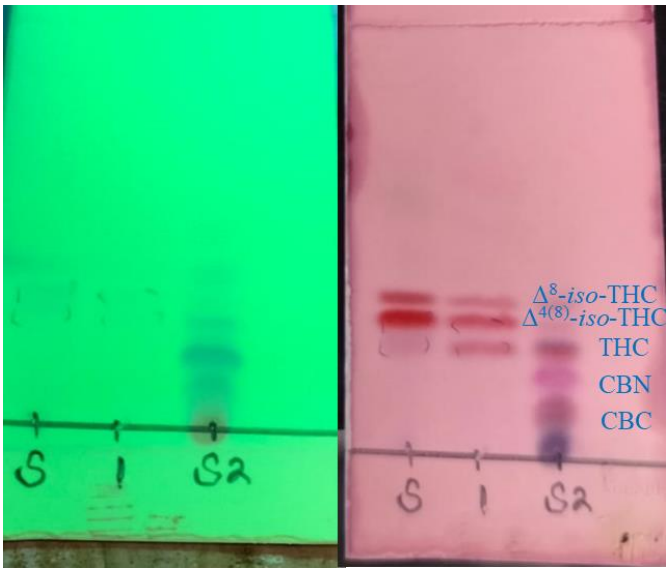

Figure 4. TLC profiles of compounds isolated from the leaves of *C. sativa*

The assumption on the presence of the phenolic group increasing antidiabetic activity became stronger when the phenolic hydroxy of THC was replaced by acetoxy moiety in acetylated THC, which showed no inhibition against α-glucosidase. Figure 5 elucidates the structure-activity relationship with the inhibitory activity of the derivatized compound.

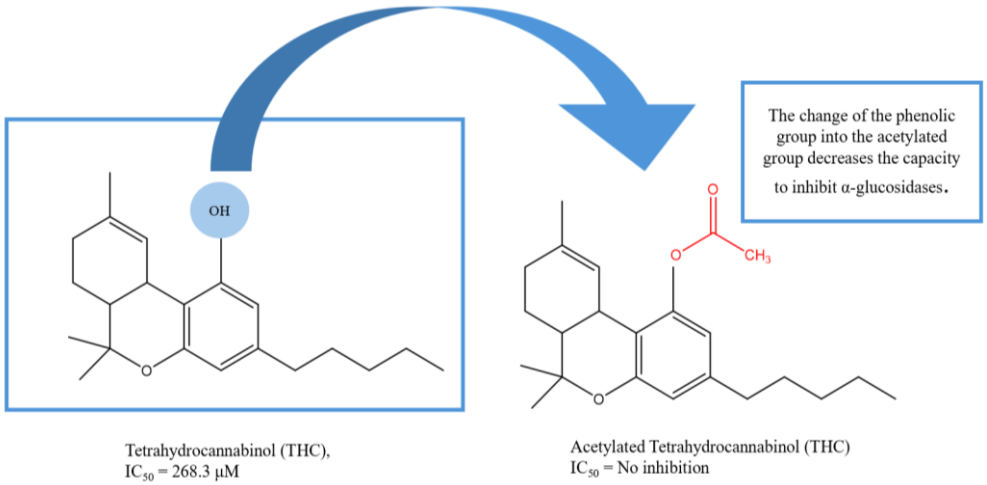

Figure 5. SAR diagram of THC with the inhibition of α-glucosidases

From these observations, it was suggested that the presence of a phenolic group as well as a proper core structure and increasing polarity increases the possibility of enhanced inhibition. Figure 6 describes the α-glucosidase inhibitory activity of all the isolated and acetylated compounds against maltase and sucrase.

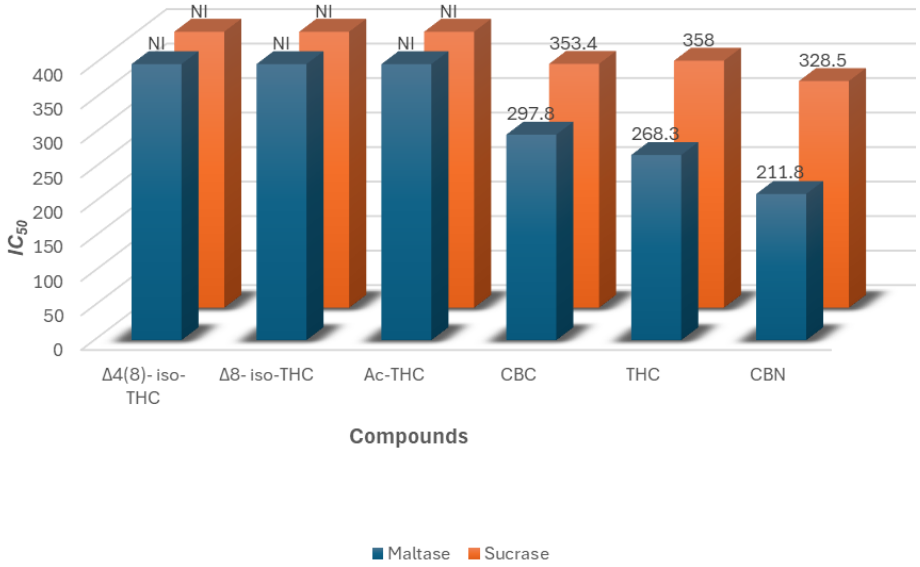

Figure 6. α-Glucosidase inhibitory activity of different compounds isolated from *C. sativa*

5. Conclusion

Five compounds were isolated from the leaves of cannabis sativa and then evaluated for their antidiabetic activity against maltase. The structure-activity relationship was described by derivatizing the phenolic group of tetrahydrocannabinol. This study concluded that the presence of phenolic moiety plays a vital role in increasing antidiabetic activity for alpha-glucosidase inhibitors. A higher number of phenolic groups results in enhanced antidiabetic activity. Replacing the phenolic group with any other group such as acetyl can consequently decrease such activity.

6. Acknowledgements

This project was supported by the Ratchadaphiseksomphot Endowment Fund through The Center of Excellence in Natural Products (CENP). Gratefulness is also extended to the Graduate School of Chulalongkorn University for the NON-ASEAN Scholarship.

7. References

Campos, A. C., Moreira, F. A., Gomes, F. V., Del Bel, E. A., & Guimaraes, F. S. (2012). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1607), 3364-3378. https://doi.org/10.1098/rstb.2011.0389

Coetzee, C., Levendal, R.-A., Van de Venter, M., & Frost, C. (2007). Anticoagulant effects of a Cannabis extract in an obese rat model. *Phytomedicine*, *14*(5), 333-337. https://doi.org/10.1016/j.phymed.2006.02.004

Derosa, G., & Maffioli, P. (2012). α-Glucosidase inhibitors and their use in clinical practice. *Archives of medical science: AMS*, *8*(5), Article 899. https://doi.org/10.5114/aoms.2012.31621

El-Remessy, A. B., Al-Shabrawey, M., Khalifa, Y., Tsai, N.-T., Caldwell, R. B., & Liou, G. I. (2006). Neuroprotective and blood-retinal barrier-preserving effects of cannabidiol in experimental diabetes. *The American journal of pathology*, *168*(1), 235-244. https://doi.org/10.2353/ajpath.2006.050500

Jin, D., Dai, K., Xie, Z., & Chen, J. (2020). Secondary metabolites profiled in cannabis inflorescences, leaves, stem barks, and roots for medicinal purposes. *Scientific Reports*, *10*(1), Article 3309. https://doi.org/10.1038/s41598-020-60172-6

Kovalchuk, O., & Kovalchuk, I. (2020). Cannabinoids as anticancer therapeutic agents. *Cell Cycle*, *19*(9), 961-989. https://doi.org/10.1080/15384101.2020.1742952

Nguyen, A.-K., & Phuwapraisirisan, P. (2024). A new furanonapthoquinone from the stems of Thunbergia laurifolia. *Natural Product Research*, *38*(3), 477-485. https://doi.org/10.1080/14786419.2022.2128343

Proença, C., Freitas, M., Ribeiro, D., Oliveira, E. F., Sousa, J. L., Tome, S. M., ... & Fernandes, E. (2017). α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. *Journal of enzyme inhibition and medicinal chemistry*, *32*(1), 1216-1228. https://doi.org/10.1080/14756366.2017.1368503

Ramadhan, R., & Phuwapraisirisan, P. (2015). New arylalkanones from Horsfieldia macrobotrys, effective antidiabetic agents concomitantly inhibiting α-glucosidase and free radicals. *Bioorganic & medicinal chemistry letters*, *25*(20), 4529-4533. https://doi.org/10.1016/j.bmcl.2015.08.069

Salehi, B., Ata, A., V. Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., Abdulmajid Ayatollahi, S., Valere Tsouh Fokou, P., Kobarfard, F., & Amiruddin Zakaria, Z. (2019). Antidiabetic potential of medicinal plants and their active components. *Biomolecules*, *9*(10), Article 551. https://doi.org/10.3390/biom9100551

Salim, B. (2005). Diabetes mellitus and its treatment. *International Journal of Diabetes and Metabolism*, *13*(3), 11-134. https://doi.org/10.1159/000497580

Suttithumsatid, W., Shah, M. A., Bibi, S., & Panichayupakaranant, P. (2022). α-Glucosidase inhibitory activity of cannabidiol, tetrahydrocannabinol and standardized cannabinoid extracts from Cannabis sativa. *Current Research in Food Science*, *5*, 1091-1097. https://doi.org/10.1016/j.crfs.2022.07.002

Velasco, G., Carracedo, A., Blázquez, C., Lorente, M., Aguado, T., Haro, A., Sánchez, C., Galve-Roperh, I., & Guzmán, M. (2007). Cannabinoids and gliomas. *Molecular neurobiology*, *36*, 60-67. https://doi.org/10.1007/s12035-007-0002-5

Vučković, S., Srebro, D., Vujović, K. S., Vučetić, Č., & Prostran, M. (2018). Cannabinoids and pain: new insights from old molecules. *Frontiers in pharmacology*, *9*, 1259. https://doi.org/10.1007/s12035-007-0002-5

Weiss, L., Zeira, M., Reich, S., Har-Noy, M., Mechoulam, R., Slavin, S., & Gallily, R. (2006). Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. *Autoimmunity*, *39*(2), 143-151. https://doi.org/10.1080/08916930500356674