CHAPTER II
LITERATURE REVIEW

2.1 *Cannabis sativa L.*

Figure 2.1 Morphology of *Cannabis sativa L.* (Geogre Mouratidis, 2020).

Cannabis sativa L. (Figure 2.1) is a kind of plant with different names like cannabis, hemp, and marijuana. It is thought to have originated from central Asia (Voeks, 2014), but now it has widespread cultivation by cosmopolitan distribution. The composition of *C. sativa* has been found to include several bioactive cannabinoid molecules with the main components that are indicated in Figure 2.2. However, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have been focused on in pharmacy to treat some diseases like cancer and Autism Spectrum Disorder, and for inclusion in dietary supplements, food, and beverages. THC is a psychoactive compound, while CBD is non-psychoactive, and a correct ratio between these molecules is needed for high effectiveness for treatment (Table 2.1).

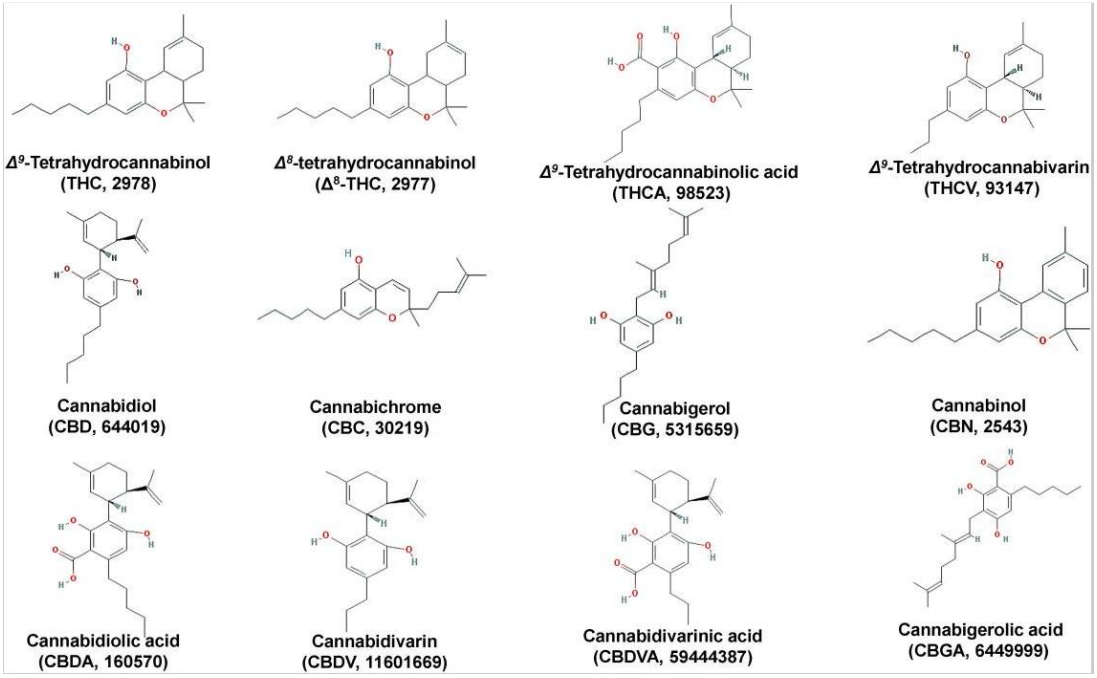

Figure 2.2 The main components of *Cannabis sativa L.* extract (Aliferis and Bernard-Perron, 2020).

Supporting medical purposes, *C. sativa* is growing in Suranaree University of Technology. Cannabis can be harvested and purified to collect THC and CBD for some applications. The advantage of CBD and THC are shown in Tables 2.1 and 2.2. However, CBD and THC are not very soluble in water. The glycosylation can be applied to change to derivatives that are highly soluble in water. Scientists have tried to look for enzymes that can help in glycosylation. In *S. rebaudiana*, 44 uridine diphosphate glucose glucosyltransferase (UGTs) enzymes have been found for glycosylation with glucose (Zhang et al., 2020). The details of *S. rebaudiana* are described in the next section.

Table 2.1 Medical characteristics of cannabis.

Medicine	Medical characteristics	References
CBD: THC Ratio (20:1)	Pediatric epilepsy treated 74 patients were 2016 (age range 1-18 years)	(Tzadok et al., 2016)
CBD:THC (20:1) twice a day	Autism Spectrum Disorder and epilepsy	(Ponton et al., 2020)
CBD (combining 5 ug/ml CBD and 4 gray of radiation)	Cancer treatment (in vitro)	(Yasmin-Karim et al., 2018)
CBD: THC Ratio (1:1)	Attention-deficit hyperactivity disorder (ADHD)	(Black et al., 2019)

Table 2.2 Cannabis in cosmetic, dietary supplement, food and beverages.

APPLICATION	REFERENCES
- Coca-Cola are planning to supplement some of their products with CBD; cosmetic industry used hempseed oil as a sun cream due to UV absorption and high vitamin E content; hemp oil was used as cooking oil like olive oil, which has established benefits for cardiovascular health; dietary supplement	(Cerino et al., 2021)
- Dietary supplement	(Corroon et al., 2020)
- Cannabidiol coffee	(Monshouwer et al., 2011) (Wouters et al., 2012)
- Acne treatment	(Spleman et al., 2018)

2.2 Characterization of cannabinoids (THC and CBD)

The aim of cannabinoids characterization is to provide pharmacokinetic information on cannabinoids when fed in oral administration. It has four parts absorption, distribution, metabolism, and excretion.

2.2.1 Absorption

In oral administration, absorption of cannabinoids involves transport across membranes of the epithelial cells in the gastrointestinal tract. The first organ

is the stomach, which has a relatively large epithelial surface, but its thick mucous layer and short transit time limit cannabinoid absorption. The cannabinoids are sent to the small intestine, where cannabinoids can absorb into the small intestine epithelium as shown in Figure 2.3. The absorption of exogenous molecules in the small intestine occurs by transcellular transport, active transport, facilitated diffusion, receptor-mediated endocytosis, paracellular transport and pinocytosis (Boyle, 2005). However, the cannabinoids are nonpolar and can diffuse directly across the membrane, followed by transcellular transport into the bloodstream (Wakshlag et al., 2020).

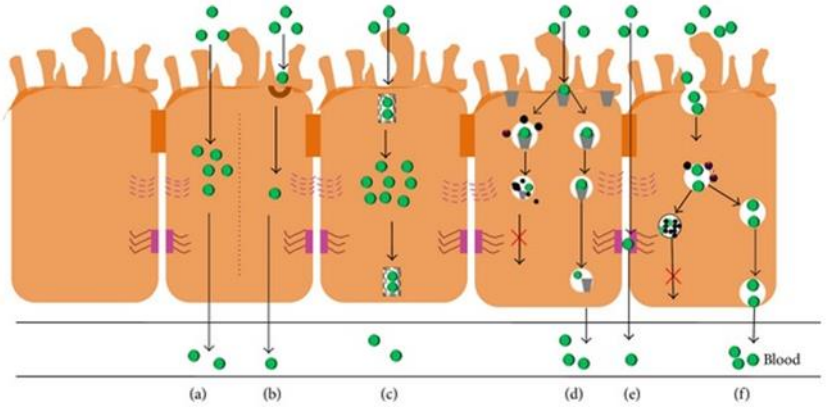

Figure 2.3 The absorption of molecules in the small intestine. (a) Transcellular transport; (b) active transport; (c) facilitated diffusion; (d) receptor-mediated endocytosis; (e) paracellular transport; (f) pinocytosis (Xu et al., 2013).

2.2.2 Distribution

After cannabinoids absorb into the bloodstream, the cannabinoids are distributed to everywhere in our body. Based on the property of cannabinoids, they can interact with blood proteins like plasma albumin to move in the circulatory systems. In an effective amount, cannabinoids will target organs to work in the human endocannabinoid system. In the previous study, 97% of the cannabinoid bound to the plasma protein and was distributed to the circulatory system (Millar et al., 2018). Depending on the specific molecule, cannabinoids can be strongly bound or weakly bound. When bound with albumin, another cannabinoid can bind to

albumin and free THC or CBD. THC is highly lipophilic and can diffuse across membranes based on concentration gradients when unbound. Cannabinoid transporters pump THC back into the plasma, limiting access to the blood-brain barrier. In our body, the system barrier has an assignment to protect some organs like the blood-brain barrier, blood - testicular barrier and blood – placental barrier (Figure 2.4).

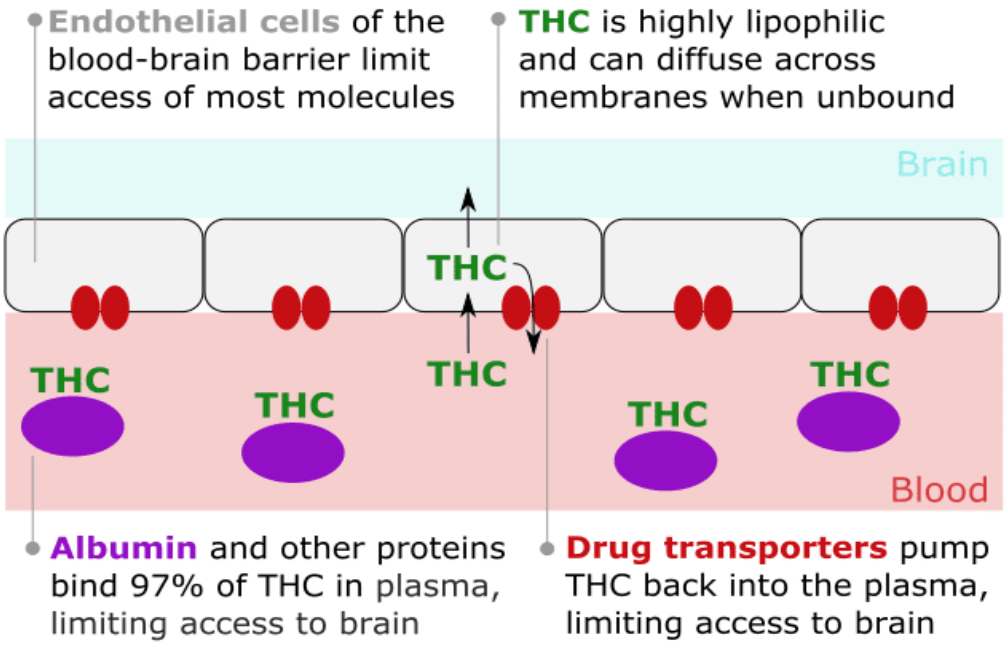

Figure 2.4 Distribution of THC in the bloodstream (Trad-Paulo Gómzer, 2017).

2.2.3 Metabolism

Metabolism of cannabinoids includes several chemical reactions that change the structure of cannabinoids to another molecule, which can be activated or inactivated the cannabinoids and can target them for excretion. Cannabinoids are carried in the blood directly to the liver, where they will be metabolized in some manner. This is called the first-pass metabolism, referring to the first pass of cannabinoids through the liver and this will typically greatly reduce the bioavailability of cannabinoids. In certain cases, metabolism in the liver actually activates cannabinoids, but this is less common, and the first pass effect can inactivate over

90% of orally administered cannabinoids before they are able to reach the general circulation. This must be taken into account when determining the appropriate dosage.

In this case, cannabinoids will react with cytochrome P450 in the liver and change to another molecule, as summarized in Figures 2.5 and 2.6.

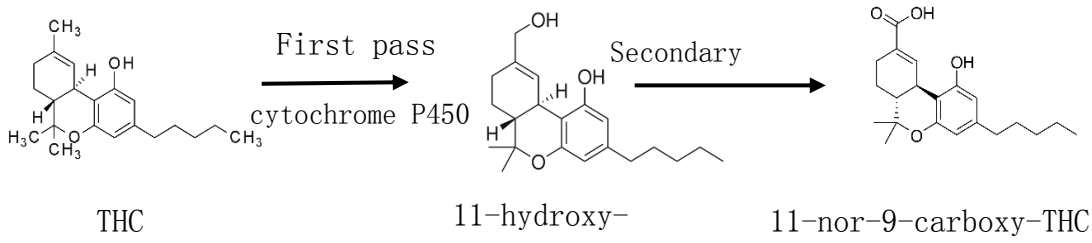

Figure 2.5 Metabolism of THC (McGilveray, 2005).

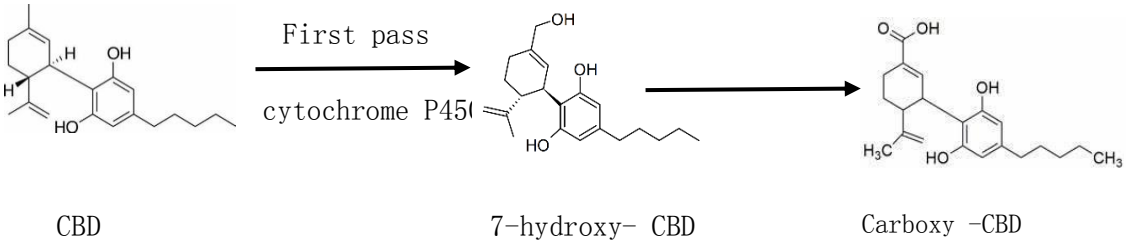

Figure 2.6 Metabolism of CBD (Wakshlag et al., 2020).

2.2.4 Excretion

Finally, cannabinoids will exit the body by urination, defecation, exhalation and sweating, which depends on the administration of cannabinoids. Normally with oral administration, the kidneys are heavily involved in this process, as they must remove poisonous substances from the bloodstream (Rimmington, 2020).

2.3 Endocannabinoid system

The endocannabinoid system is a biological system of endocannabinoids, it includes the receptors CB1 and CB2, which are G protein-coupled receptors, endocannabinoids (2-arachidonoyl-glycerol (2-AG), anandamide (AEA), the enzymes for synthesis of endocannabinoids (diacylglycerol lipase (DAGL), N-acylphosphatidyl-

ethanolamine phospholipase D (NAPE-PLD)), degradation (fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) (Kienzl et al., 2020 and Cristino et al., 2020). The endocannabinoid system is a prevalent neuromodulatory system that has significant roles in central nervous system development, synaptic plasticity, immune cells, and the response to endogenous and environmental insults (Lu and MacKie, 2016)

Phytocannabinoids are the naturally occurring cannabinoids found in the cannabis plant such as tetrahydrocannabinol (THC) and cannabidiol (CBD). They also can activate the receptors CB1 and CB2, and have similar effects to endocannabinoids.

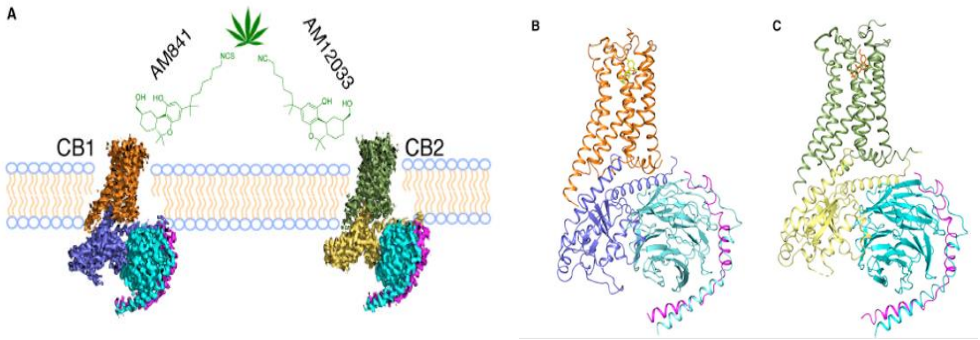

Figure 2.7 Structures of CB1 and CB2 receptors (Hua et al., 2020).

2.3.1 Central nervous system

In response to increased intracellular Ca^{2+} concentration, both endocannabinoids are produced on demand. AEA is synthesized from N-acyl-phosphatidylethanolamine (NAPE) by NAPE-specific phospholipase D (NAPE-PLD) and 2-AG is produced from diacylglycerol (DAG) by DAG lipase (Zou and Kumar, 2018). After synthesis, endocannabinoids are released into the intracellular space. Because endocannabinoids are uncharged hydrophobic molecules, they cross directly through the membrane based on concentration gradients (Boyle, 2005). Endocannabinoids will bind to the receptor CB1, and it will activate the transient receptor potential cation channel subfamily V member 1 to inhibit L-type Ca^{2+} channels. Activated CB1 will then inhibit neurotransmitter release through the suppression of calcium influx. The functions of the endocannabinoids in the central nervous system include memory, motor coordination, pain perception, feeding, appetite, and coping with stress, and it is related to the defects schizophrenia and epilepsy (Fride, 2005). The CB2 receptor has

been identified at lower levels in the brain (Zou and Kumar, 2018). Intracellular CB2 inhibits neuronal firing in medical prefrontal cortical pyramidal neurons by activating Ca^{+2}-activated chloride channels, indicating that it is involved in the regulation of neuronal activity (Zou and Kumar, 2018).

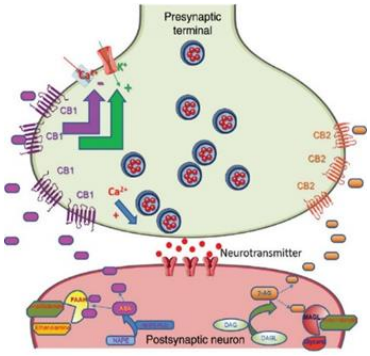

Figure 2.8 Endocannabinoid system.

2.3.2 Immune endocannabinoid system

Immune cells have many types such as CD^{8+} T cells, CD^{4+} T cells, B lymphocytes, NK cells, neutrophils, eosinophils, mast cells, monocytes, tumor-associated macrophages, dendritic cells and myeloid-derived suppressor cells as is shown in Figure 2.9 (Kienzl et al., 2020). With different effects on immune cells, the immune-endocannabinoid system contributes to the balance between neuroinflammation and neurodegeneration in the human body (Tanasescu and Constantinescu, 2010).

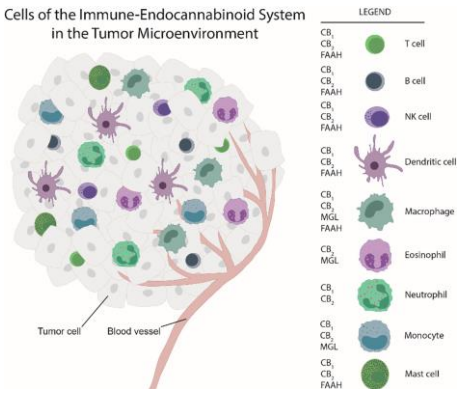

Figure 2.9 The immune endocannabinoid system (Kienzl et al., 2020).

Moreover, due to the barrier complexity of some parts of the human body, conventional drugs cannot easily be distributed and absorbed into targeted tissues. In the instance of the blood-brain barrier, the combination of cannabidiol (CBD) and tetrahydrocannabinol (THC) was used as a medicine for brain treatment, such as autism spectrum disorder, and epilepsy (Ponton et al., 2020), and attention-deficit hyperactivity disorder (Black et al., 2019). However, the drug transporter can pump THC back into the plasma, which limits it to the brain (Calapai et al., 2020).

2.4 Glycosides

In chemistry, a glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. This linkage is broken by acid or enzyme hydrolysis and both glycone and aglycone parts are separated. The glycone part is water-soluble but insoluble in organic solvents, whereas aglycone part may have opposing properties. They are formed by the biochemical glycosylation reaction which makes water-insoluble compounds more polar and the water-soluble. In the glycosides, the classification of glycosides can be based on linkage, based on nature of glycone, based on nature of aglycone and based on therapeutic activity (Mistry, 2021). For examples based on linkage, glycosides are separated into C-glycosides, O-glycosides (Figure 2.10), S-glycosides, and N-glycosides.

The catalyst for glycoside formation is acid, base, or enzyme, which have been shown as examples in Table 2.4. However, enzymatic synthesis has proven itself to be a promising alternative to the laborious chemical synthesis of glycosides by avoiding the necessity of numerous protecting group strategies needed in chemical synthesis because the reactions often lacking regio- and stereoselectivity (Hayes et al., 2017, Mestrom et al., 2019). The synthesis for the glycoside prodrugs of daunorubicin with chemical catalysts provides an instance of the laborious procedure required (De Graaf et al., 2003). The daunorubicin prodrug was synthesized in four reactions with many catalysts and reactants including (i) Ag_2O-CH_3CN; (ii) $NaBH_4$, $CHCl_3$-iPrOH; (iii) DSC-Et_3N, CH_3CN; (iv) DMF-Et_3N; and (v) MeOH-MeONa (Ghosh et al., 2000). In another example of use of chemical catalysts, the synthesis of glycosyloxymethyl prodrugs had two steps with many chemical catalysts that were described in Elferink et al., 2022.

Based on natural glycosylation, the synthesis of glycosides is easily accomplished by glycosyltransferases and transglycosidases. With curcumin glycosides, a glucosyltransferase from *Phytolacca americana* and a cyclodextrin glucanotransferase (a transglycosidase) from *Bacillus macerans* were used to synthesize curcumin D-glucoside and curcumin oligosaccharides, respectively (Wang, 2009). Natural glycoside production is most extensively done in plants in which glycosylation is catalyzed by uridine diphosphate (UDP) glycosyltransferases (UGTs), primarily from family 1 of glycosyltransferases (Wang, 2009). These plant UGTs catalyze glycosyl transfer via a direct displacement S_N2-like mechanism, similar to other inverting glycosyltransferases (GTs) (Wang, 2009). Scientists have found a large number of UGT enzymes that have evolved for the glycosylation of plant natural products. Those enzymes have been applied to produce cannabinoid glycosides (Hardman et al., 2017), rebaudioside D (L. Chen et al., 2020), and so on. Rebaudioside D was formed by a multiple enzyme system including UGT76G1, UGTSL2, and StSUS1 (L. Chen et al., 2020). In this case, UDP-Glc as a sugar donor was synthesized by *Solanum tuberosum* sucrose synthase (StSuSy1) from UDP and sucrose (Schmölzer et al., 2016). The cost of production was reduced due to the recycling of UDP to UDP-Glc by StSuSy1. Another example is *Arabidopsis thaliana* UGT73C6, which catalyzes the 23-O-glucosylation of brassinosteroids, so it has potential for glycosylation of steroids and other hydrophobic drugs. Brassinosteroids are steroid hormones of plants that play important roles in plant growth and development (Gan et al., 2021). Some other enzymes were mentioned in previous reviews (Wang, 2009), such as UGT71G1 from *Medicago truncatula* for saponin biosynthesis and UGT72L1 from *Medicago* for epicatechin 3'-O-glucoside synthesis.

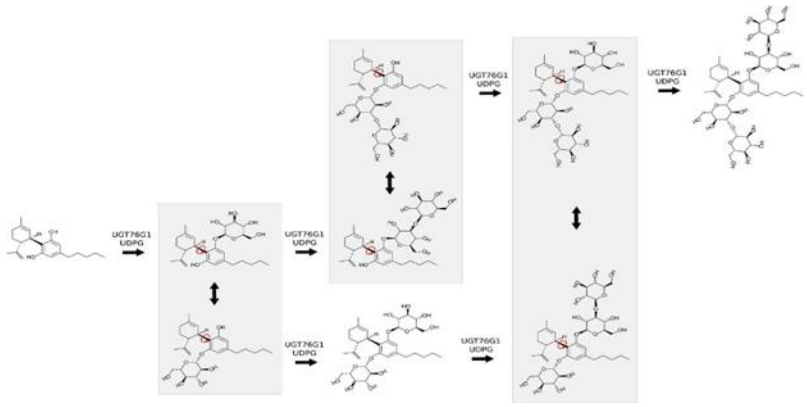

Figure 2.10 The pathway of CBD glycosides using UGT76G1 enzyme (Hardman et al., 2017).

2.5 Advantage of CBD glycosides prodrug

2.5.1 Targeting and the role of carbohydrate in cellular interactions

Previous studies have shown that carbohydrates play a significant role in cellular interactions, such as cellular signaling, recognition and interaction of cells with each other and the extracellular environment (Martin et al., 2022, Brandley and Schnaar, 1986, Shchegravina et al., 2021). That is a reason for the application of carbohydrates to drug delivery systems. It can gain many advantages, such as remarkable selectivity for protein receptors, simple structure for determination, being biocompatible and biodegradable materials, there being many different natural carbohydrate sources, shielding therapeutic agents or nanoparticles from undesirable interactions with proteins, and being highly soluble, leading to improved drug administration (Shchegravina et al., 2021). The most prominent are targeting delivery systems based on monosaccharide ligands such as D-glucose, D-galactose, D-quinovose, D-xylose, D-fucose, L-arabinose, and so on (Elferink et al., 2022) and carbohydrate nanoparticles. The prodrugs targeting the organ depend on the kind of monosaccharide, which uses the SLC2A family of transporters (Shchegravina et al., 2021). For example, the brain is targeted by glucose due to GLUT1, which is highly expressed on the surface of the cerebral capillary lumen; the liver is targeted by mannose or galactose due to the asialoglycoprotein receptor (ASGP-R), the lung is targeted by mannose or glucose due to macrophages which are abundant in lung

tissues, that have a mannose receptor on the surface; and GLUT1 is highly expressed in lung cancer issues (Chen et al., 2020). By the same principle of receptor binding, CBD glycosides are prodrugs with many advantages thanks to monosaccharides.

2.5.2 Enhanced Pharmacokinetic properties

Pharmacokinetic properties are presented in four parts: absorption, distribution, metabolism, and excretion.

2.5.2.1 Absorption

In oral administration, absorption of CBD glycosides involves transport across the membranes of epithelial cells in the gastrointestinal tract. The stomach is the first organ, with a comparatively large epithelial surface area; yet, absorption of CBD glycosides is limited by its thick mucous layer and fast transit time. Then, the CBD glycosides are sent to the small intestine, where they can be absorbed into the small intestine or large intestine. Addition of the carbohydrate group of glucose in CBD glycosides can increase the solubility of CBD due to the OH groups of sugar, which can increase the bioavailability of drugs. In the case of cannabidiol (CBD), which is a hydrophobic molecule with low bioavailability in oral administration. CBD has been glycosylated with glucose by UGT76G1, and based on the amount of glycosylation, the aqueous solubility of CBD glycoside increases, leading to an increase in the bioavailability of CBD glycoside (Hardman et al., 2017). In other research, they tested with high bioavailability assay of CBD glycoside (VB110-two glycoside of two-side OH group) on eight-week-old male Swiss mice. The results were found CBD and CBD-glycosides in the large intestinal at 60 min and 90 min time points. It was hydrolyzed by beta-glycosidases from the large intestinal microflora (Zipp et al., 2017). In addition, the highly aqueous solubility of CBD glycosides also enables new formulations for delivery in transdermal or aqueous formulations. In patents, CBD glycoside was used in numerous formulations, including gel, gel-like compositions, ointments, creams, patches, lotions, sprays, foam, and oils (Stinchcomb et al., 2010). Therefore, there is beginning to show promise for the use of CBD glycosides in improving human health.

2.5.2.2 Distribution

After CBD glycosides absorb into the bloodstream, they are distributed everywhere in our body. Based on the aqueous solubility of CBD glycosides, they can easily move in the circulatory system and target tissues with

increased expression of glycosidase. Especially, various glycosidase enzymes are also overexpressed in different cancer types, such as β-glucosidase (breast, gastric, and liver); α-fucosidase (ovarian, gliomas, colon, pancreatic), and so on (Martin et al., 2022). In addition, in cancer cells, the glucose transporters (GLUTs) are overexpressed due to the higher uptake of glucose than in normal cells. Therefore, CBD glycoside constitute a promising design for supporting cancer treatment.

In a previous study, 97% of the cannabinoid was bound to the plasma protein and distributed to the circulatory system due to its hydrophobic properties, which led to low bioavailability (Millar et al., 2018). With glucose moieties, CBD glycosides can also cross through some barriers in our body by intranasal, stereotactic, or intrathecal delivery and can also pass the blood-brain barrier with the sugar facilitating the transport (Zipp et al., 2017). After glucose moieties support crossing the blood-brain barrier, the glycosidase in the brain can break the glycosidic bond to release CBD (Zipp et al., 2017).

2.5.2.3 Metabolism and excretion

CBD glycosides are prodrugs with low degradation or metabolism in the stomach and small intestine, which leads to higher total bioavailability of CBD glycoside upon oral delivery. It can be found in the small and large intestines, where some molecules were degraded by beta-glycosidases from microflora [17, 22]. However, in first-pass metabolism CBD glycosides pass through the liver and react with CYP450 for detoxification and targeting them for excretion. Nevertheless, glycosylation of acceptor hydroxyl groups may afford protection, preventing CBD glycosides from binding to the CYP450 active site (Zipp et al., 2017). Therefore, long-term release of CBD formulation from CBD glycosides supports reduced toxicity in the liver and slow excretion from the body. In addition, the glucose moiety with several possible numbers of glucose moieties on both OH groups becomes more varied and complex, and when CBD glycosides are administered, the CBD glycosides prodrug delivery kinetics are altered, resulting in a prolonged-release drug formulation.

2.5.3 Reduced toxicity and side effects

This property of low toxicity is also related to targeting cells because CBD glycosides are a kind of inactive drug if their parent drugs are not released, so

target cells overexpressing the appropriate glycosidase are most affected. After release from the parent drug, the glucose moiety has many advantages, such as nontoxicity, no immunogenicity, good biocompatibility, and biodegradation, as mentioned in a previous paper (Chen et al., 2020).

Based on the low toxicity and availability of targeting cancer cells by glycosidase and properties of high GLUTs expression in cancer cells, CBD glycosides are a promising modern medicine for cancer cell treatment. Besides that, the improved solubility of CBD glycoside leads to high bioavailability. The amount of drug dose can be reduced, and there are diverse routes of medication administration when compared with traditional drugs.

In addition, conventional cancer drugs affect not only cancer cells but also normal cells. Thus, lack of specificity to tumor cells and high toxicity still exist in conventional drugs. Therefore, the new drug delivery is very necessary and challenging for scientists to improve the properties of conventional drugs. In this case, glycoside prodrugs not only significantly improved pharmacokinetics but also further enhanced the permeability, solubility, stability, specificity, and selectivity of GDs to target cells. With GDs, the following benefits can be gained: cell targeting based on sugar; high solubility leading to high bioavailability; low toxicity due to the sugar group; and high biocompatibility (Martin et al., 2022) (Chen et al., 2020). More applications of glycosylation in pharmacy, food/dietary supplements, cosmetics are summarized in Table 2.3.

Table 2.3 Glycoside prodrugs for modern medicine and other applications.

Glycoside prodrugs	Carbohydrate group(s)	Catalyst used for synthesis or source	Activating enzyme	Function of GD	References
CBD glycosides	Glucose and glucooligosaccharides	Enzymes catalyst	Beta-glycosidase	Anti-psychotic, a neuroprotectant, and other maladies	(Zipp et al., 2017)
Curcumin glycosides	Gluco-oligosaccharide	Enzymes catalyst	Glycosidase	Anticancer, anti-inflammatory, neuroprotective (Alzheimer's disease anti-oxidative affection)	(Hamada et al., 2020)
Digoxin and lanatoside C	Glycone	Medicinal plant	-	Anti-neuroinflammation	(Jansson et al., 2021)
Glycosylated Paclitaxel	Glucose	Chemical catalyst	β-glucuronidase	Anticancer for breast cancer and ovarian cancer cell	(Mao et al., 2018)
ETP, a topoisomerase II inhibitor	Glucosyl acetone-based ketel-linked glycoside prodrugs	Chemical catalyst	β-glucuronidase and acid-triggered ketal hydrolysis	Cancer therapy	(Yu et al., 2020)

Table 2.3 Glycoside prodrugs for modern medicine and other applications (Continuous)

Glycoside prodrugs	Carbohydrate group(s)	Catalyst used for synthesis or source	Activating enzyme	Function of GD	References
Glycosyloxymethyl prodrugs with 5-fluorouracil, thioguanine, propofol and losartan.	Monosaccharides (β-D-glucose, β-D-quinovose, β-D-xylose, β-D-galactose, β-D-fucose, α-L-arabinose	Chemical catalyst	β-glycosidases in the GI-tract	Anticancer for acute myeloid leukemia, acute lymphocytic leukemia, and chronic myeloid leukemia; Treatment of irregular heart rate and high blood pressure	(Elferink et al., 2022)
Glycoside prodrugs of daunorubicin	Glucose and galactose	Chemical catalyst	β - glycosidases	Cancer therapy	(De Graaf et al., 2003)(Ghosh et al., 2000)
Rebaudioside D	Glucose	Enzyme catalyst	-	Diabetics (food supplement)	(L. Chen et al., 2020)
Myricitrin glycosides	Galactose	Enzyme catalyst	-	Anti-oxidative activity	(Shimizu et al., 2006)

Table 2.3 Glycoside prodrugs for modern medicine and other applications (Continuous)

Glycoside prodrugs	Carbohydrate group(s)	Catalyst used for synthesis or source	Activating enzyme	Function of GD	References
Glycosylated antioxidants, Mangiferin glucosides	Glucose, maltose	Enzyme catalyst	-	Antioxidants	(De Winter et al., 2015), (Wu et al., 2021)
Quercetin glucosides	α- Oligoglucosylation	Enzyme catalyst	α – glucosidase	Flavonoid as antioxidant	(Murota et al., 2010)
Menthol glucoside	Glucose	Enzyme catalyst	-	Hair lotions, oral hygiene, dental, skin care product, skin irritation, so on.	(Kurze et al., 2021)
Glycosylated-chitosan derivaties	oligosaccharides	Chemical catalyst	-	Apply in different sectors such as food supplement, biomedical, so on.	(Sacco et al., 2020)

2.6 *Stevia rebaudiana*

Figure 2.11 *Stevia rebaudiana* (Madan et al., 2010).

Stevia rebaudiana (Figure 2.11) is a plant with sweet taste. It can also be called honey leaf or sweet leaf. It produces sweet taste but no energy values. Normally, it can be used instead of sugar for reducing dental caries in children and reducing digestible sugar in the diet. In addition, the compounds of *S. rebaudianna* have many advantages, including anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, etc, (Ferrazzano et al., 2016). The biosynthesis pathway is shown in Figure 2.12. The *S. rebaudiana* has been investigated and found to have 44 UDP-glucosyltransferase (UGTs), which are displayed in the phylogeny tree in Figure 2.13. There are many UGTs, three of which have been identified and characterized (srUGT85C2, srUGT74G1 and SrUGT76G1) (Richman et al., 2005). Of the 44 UGT enzymes, the SrUGT76G1 enzyme with favorable characteristics has been applied to generate several products, such as sweeteners with good taste which replace sugar while providing low energy (rebaudioside A, rebaudioside C), curcumin glycosides and cannabinoid glycosides with improved properties compared to the original molecules, as shown in Table 2.4.

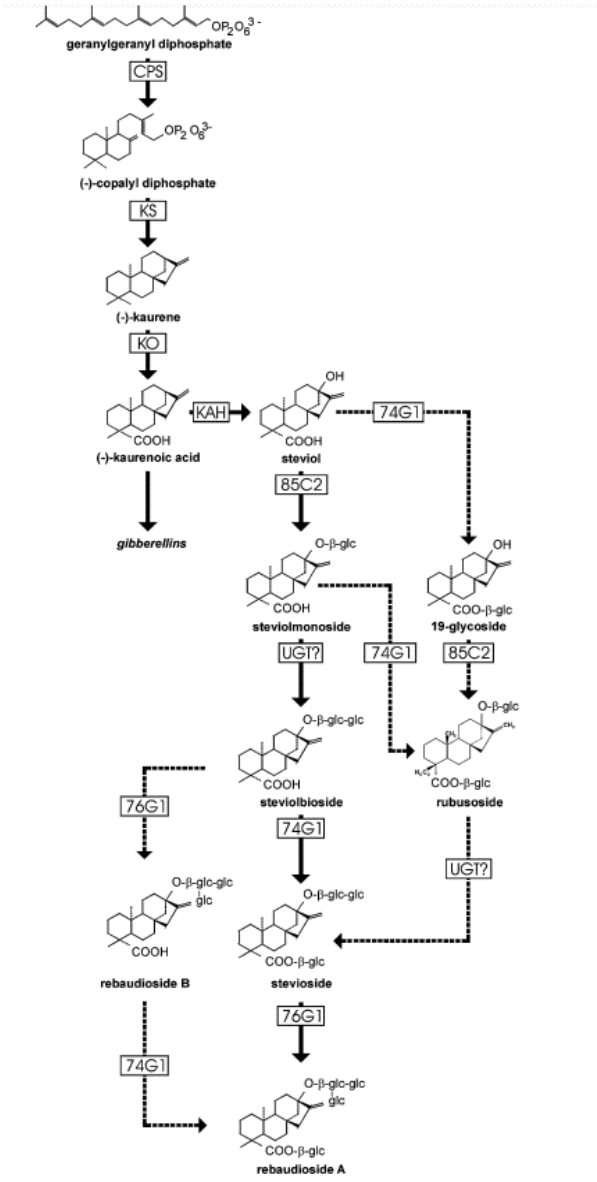

Figure 2.12 Biosynthesis pathway of the steviol glycosides. Abbreviations: CPS, copalyl diphosphate synthase; KS, kaurene synthase; KO, kaurene oxidase; KAH, kaurenoic acid oxidase; UGTs are abbreviated to their numerical identifiers (Humphrey et al., 2006).

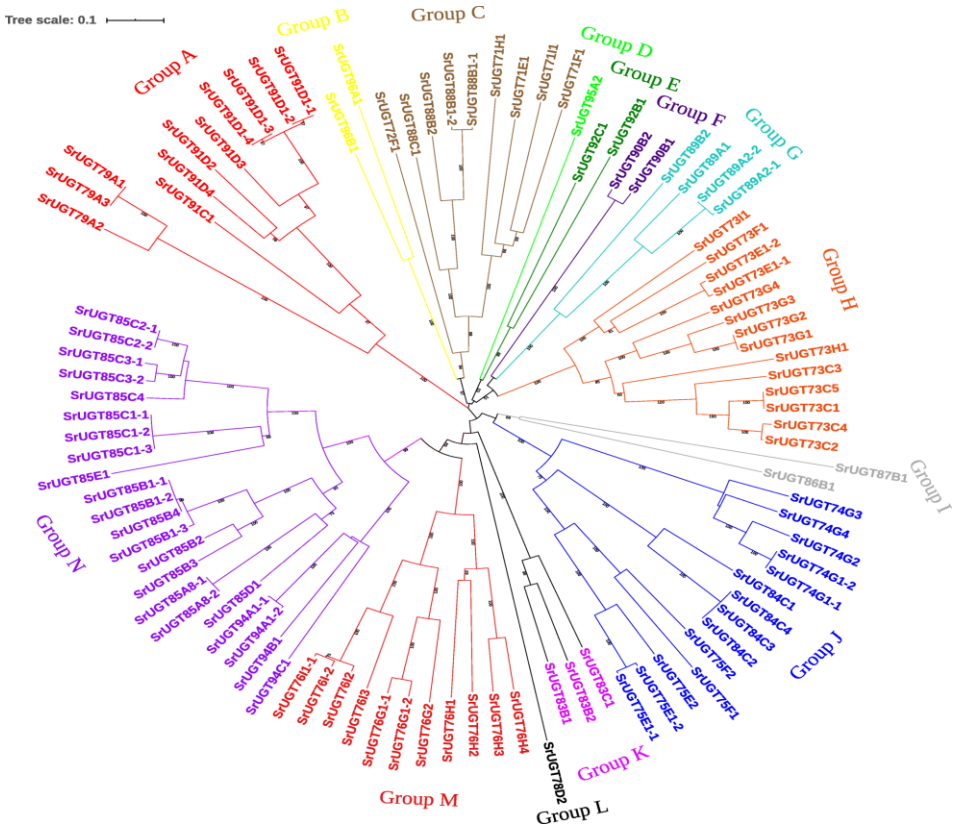

Figure 2.13 The phylogeny tree of UGTs glycosyltransferases in *Stevia rebaudiana* (Zhang et al., 2020).

Table 2.4 Summary application of SrUT76G1 for glycosylation.

Enzymes	Host cells	Products	References
SrUGT76G1 (*Stevia rebaudianna*) and Os03g0702000p (*Oryza sativa)*	*E. coli* BL21(DE3)	Cannabinoid glycoside	(Hardman et al., 2017)
SrUGT76G1, UGT74G1, and UGT85C2	*BL21-CodonPlus(DE3) E.coli* (Stratagene)	Rebaudioside A	(Richman et al., 2005)
UGT-76G1*Sr*	*E. coli* BL21(DE3) cells (Novagen)	Curcumin glycoside	(Dewitte et al., 2016)
SrUGT76G1	*E. coli* BL21(DE3)-derived Rosetta strain	Rebaudioside C	(Kim et al., 2019)
SrUGT76G1	*E. coli* BL21(DE3) pLysS	Rebaudioside A	(Madhav et al., 2013)

2.7 Sucrose synthase (SuSy) *(Glycine max)*

Figure 2.14 Glycine max (Fraw-doktor, 2016).

Due to expense of UDP-glucose, sucrose synthase (SuSy) has been applied to catalyze the reversible transfer of a glucosyl moiety between fructose and uridine diphosphate (UDP) (sucrose + NDP <-> UDP-Glc + fructose). *Glycine max* (Figure 2.14) is commonly called soybean and has many interesting enzymes that can be applied in production of glycosides. Of these, sucrose synthase is of interest in the synthesis of glycosides. The coupled enzymes system with sucrose synthase has been applied in many reports to produce glycosides, such as hyperoside (Pei et al., 2017), ginsenoside (Dai et al., 2018), and calycosin-7-O-β-D-glucoside (Hu et al., 2020). Based on pH values between 5.5 and 7.5, the reaction can be controlled to produce UDP-Glc. For UDP recycling, the concentration of UDP could be lower than 0.5 mM and UDP inhibited the reaction when it was higher than 4 mM (Schmölzer et al., 2016). Coupled UGT and SuSy for regeneration of UDP-Glc can maximize glycoside production by raising the acceptor concentration instead of a further reduction of UDP.

2.8 Fermentor

Figure 2.15 5 L fermentor and 50 L fermentor systems.

The fermentor (Figure 2.15) is a kind of equipment used for cell cultivation with probes and sensors to monitor cell cultivation conditions. Several parameters can be controlled in fermenters, such as the rate of rotation of stirring paddles which provides homogenization of the medium with cells and distributes heat and air for cells; the flow of compressed air into the chamber via the aerator, the use of a defoamer to prevent the formation of foam; and the pH, DO, and temperature which are monitored with probes to provide good conditions for cell growth. In the case of recombinant protein production such as in *E. coli*, the two periods that are used for the optimal generation of recombinant protein are when biomass is rapidly accumulated during the non-inducing period and when protein production is achieved during the inducing period. Nevertheless, a high cell density can frequently result in a number of serious issues, such as plasmid loss from *E. coli*, a large pH drops due to cell metabolites, and limited dissolved oxygen availability. These issues frequently lead to minimal or even no protein production while maintaining a high cell density (Sivashanmugam et al., 2009). Therefore, the cell density should be controlled to a level which is optimal for the protein expression period.

2.9 High Pressure Homogenizer or Microfluidics

To reduce particle size and enhance homogeneity, high-pressure homogenization (HPH) (Figure 2.16) is done with equipment that results in highly efficient generation of emulsions or suspensions. The principle of HPH is the application of high pressure to push sample fluids through a narrow gap over a very short distance. This machine can be applied in various areas, including nano-emulsions or suspensions, enzyme extraction. In a previous study, the HPH was used to breakdown the *E. coli* for enzyme extraction with conditions of 46 Mpa pressure, feed cell concentration of 100 g/L, and 10 homogenizer passes, resulting in a disruption efficiency of 99.5% (Kleinig et al., 1995). In the case of enzyme extraction, the disruption efficiency of HPH is based on pressure, cell concentration, and time of homogenizer passes (Middelberg, 2000). The advantages of HPH for enzyme extraction are its low temperature to reduce inactivation of enzymes, the ability to breakdown cell wall and reduce viscosity without the enzymes DNase and lysozyme to reduce the cost of extraction, and its ease in applying for large scale for enzymes in industry.

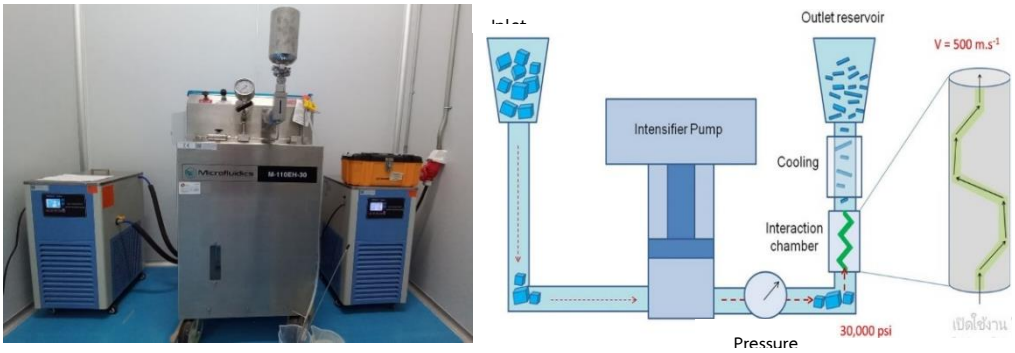

Figure 2.16 The high-pressure homogenizer.