2. REVIEW OF LITERATURE
The overall biological system depends on a complex network of chemical reactions catalyzed by specific enzymes, and any modification of the enzyme pattern may have drastic consequences for a living organism. The mechanisms by which enzymes catalyze chemical reactions are in itself one of the most fascinating fields of scientific investigation being pursued at the present time. Thus, enzymes, as catalysts, have received increasing attention from many scientific fields, especially biochemistry, biotechnology and molecular biology, microbiology, genetics, botany and agriculture, pharmacology and toxicology, medicine, and chemical engineering. Due to the complex structure and biological and physical instability of these macromolecules the mechanics of enzyme catalysis is still quite limited. Biochemical studies on enzymes provide the source for understanding how enzymes function and how the catalytic sites react with substrates. Enzymes are key components in every metabolic pathway that occurs within the cell. Thus, investigating enzymes provides valuable information about the catalytic mechanism and function in metabolism. Catalysis is intimately related to the molecular interactions that take place between a substrate and a specific part of the enzyme molecule. Today, spectroscopic methods, x-ray crystallography, and more recently, multidimensional NMR methods, provide a wealth of structural insights on which theories of enzyme mechanisms can be built.

The ability of β-glucosidases to activate glycosidic bonds renders the enzyme highly interesting as a promising biocatalyst for the metabolism of stereo/regio-specific glycosides or oligosaccharides, molecules potentially useful as functional materials, nutraceuticals, or pharmaceuticals because of their biosignaling, recognition, or antibiotic properties. Opposite to its hydrolytic activity, it may catalyze a glycosidic bond formation via either a thermodynamically controlled “reverse” hydrolysis or a kinetically controlled transglycosylation. The main advantage of using β-glucosidases over glycosyltransferase in such applications is that the former does not require any
involvement of expensive/unstable nucleotide sugars precursors as well as its wide functionality even under harsh reaction conditions (Bhatia et al., 2002).

Glycosides are compounds containing a carbohydrate and a noncarbohydrate residue in the same molecule. The carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a noncarbohydrate residue or aglycone. The carbohydrate component is called the glycone. If the carbohydrate portion is glucose, the resulting compound is a glucoside. Many of these compounds are pharmacological important molecules or possess other appealing properties.

The aglycone may be methyl alcohol, glycerol, a sterol, a phenol, etc. An acetal has two ether functions at a single carbon atom.

```
H
R—O—C—O—R
   |     
   R''
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Figure 1: A simple aglycone skeleton

The glycone can be attached to the aglycon in many different ways. The most common bridging atom is oxygen (O-glycoside), but it can also be sulphur (S-glycoside), nitrogen (N-glycoside) or carbon (C-glycoside). In general, one distinguishes between α-Glycosides and β-glycosides, depending on the configuration of the hemiacetal hydroxyl group. The majority of the naturally occurring glycosides are β-glycosides. Generally glycosides are more polar than the aglycones and as a result glycoside formation usually increases water solubility. This may allow the producing organism to transport and store the glycoside more efficiently.
2.1. Classification and distribution of glycosides

Many biologically active compounds are glycosides. The pharmacological effects are largely determined by the structure of the aglycone. Glycosides comprise several important classes of compounds such as hormones, sweeteners, alkaloids, flavonoids and antibiotics (Kren and Martankova, 2001).

Depending upon the chemical nature of the aglycone the glycosides are classified in Figure 2.

![Glycosides diagram]

Figure 2: Group-wise classification of glycosides

2.1.1. Saponin glycosides

Saponin glycosides are divided into two types based on the chemical structure of their aglycones (sapogenins). Saponins on hydrolysis yield an aglycone known as "sapogenin".
The main pathway is schematically presented in Figure 4; leading to biosynthesis of both types of sapogenins is similar and involves the head-to-tail coupling of acetate units. However, a branch occurs, after the formation of the triterpenoid hydrocarbon, squalene, that leads to steroids in one direction and to cyclic triterpenoids in the other.
2.1.2. Cyanogenic glycosides

The cyanogenic glycosides belong to the products of secondary metabolism, to the natural products of plants. These compounds are composed of an α-hydroxynitrile type aglycone and of a sugar moiety (mostly D-glucose). The distribution of the cyanogenic glycosides (CGs) in the plant kingdom is relatively wide, the number of CG-containing taxa is at least 2500, and a lot of such taxa belong to families Fabaceae, Rosaceae, Linaceae, Compositae and others. The biosynthetic precursors of the CGs are different L-amino acids, these are hydroxylated then the N-hydroxylamino acids are converted to aldoximes, these are turned into nitriles. The last ones are hydroxylated to a-hydroxynitriles and then they are glycosilated to CGs. The generation of HCN from CGs is a two step process involving a deglycosilation and a cleavage of the molecule (regulated by β-glucosidase and α-hydroxynitrilase). Vetter (2000) also reported that some plant species like cassava (*Manihot esculenta*) have the ability to produce cyanides and are strong cytotoxins, competitive inhibitors of the Fe³⁺ of the heme group. The cells detoxify them by glycosylation, i.e. by linking them β-glycosidically to sugar residues (usually glucose).

Most plants produce a small amount of cyanide associated with ethylene production between 3000-12000 plant species produce sufficient quantities of cyanogenic compounds (McMahon *et al.*, 1995). The enzymatic hydrolysis produces the aglycone and the sugar moiety. The CGs can be grouped according to chemical nature of substituents, namely aliphatic, aromatic groups and into the glycosides with a free α-hydroxynitrile. Some of these CGs are better known than the others because the carrying plant species (group) has a greater practical importance, several economically important plants are highly cyanogenetic (linamarin in *Manihot esculenta, Linum usitatissimum, Trifolium repens*, dhurrin in *Sorghum* species, amgydalin in rosaceous plants, lotaustral in *Lotus corniculatus*, etc.). The linamarin and lotaustral have a relatively broad distribution in the plant kingdom; have been demonstrated by Seigler *et al.* (1989) in the following

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plant families: Compositae, Euphorbiaceae, Linaceae, Papaveraceae and Fabaceae (Leguminosae). Conversely, it is generally true with few exceptions that only one or two characteristic glycoside will occur in a given plant family (Poaceae: dhurrin; Compositae: linamarin; Polypodiaceae: prunasin and vicianin, Rosaceae: amygdalin and prunasin). The cyanogenic compounds of plants belong undoubtedly to secondary plant metabolites which have or can have a chemotaxonomical character. The families Saxifragaceae, Rosaceae, Mimosaceae, Fabaceae, Myrtaceae, Linaceae and Euphorbiaceae are in the subclass Rosiidae, other cyanogenous families are in subclasses Ranunculidae (family Papaveraceae), in Lamiidae (families Caprifoliaceae, Sambucaceae, Oleaceae), Asteridae (family Compositae). CGs has been reported by Seigler et al. (1989) from many members of the three subfamilies of Fabaceae.

2.2. Synthesis of C-aryl glycosides

The C-aryl glycosides comprise a subclass of a broader family of naturally occurring C-glycosides that have biological activities and are resistant to enzymatic hydrolysis. Some representative members of this class of natural products that are of significant interest include galatamycinone, vineomycinone B2, and kidamycin. The C-aryl glycosides may be classified into four different subgroups based upon the orientation of the sugar residue(s) relative to the phenolic hydroxyl group on the aromatic ring (Martin, 2003).

2.3.1. Chemical synthesis of glycosides

The reactivity of glycosyl donors and acceptors is highly dependent on their anomeric activation and on the protecting groups that the sugar ring carries (Davis, 2000). Recently, Ye and Wong (2000) reported the methods to quantify the relative reactivities of glycosyl donors on the basis of experimentally obtained reaction rates to the synthesis of a small (33 member) library of trisaccharides and tetrasaccharides. A very important issue in carbohydrate synthesis is the
formation of the desired $\alpha$- or $\beta$-anomeric linkage. Some attempts have been made to direct the formation of otherwise unfavourable linkages through tethering of glycosyl donor and acceptor before formation of the glycosidic linkage. There is a great interest in the polymer-supported synthesis of oligosaccharides and glycoconjugates with potential applications in library and automated synthesis (Ye and Wong, 2000).

2.3.2. Enzymatic synthesis of glycosides

Enzymatic synthesis of glycosides is to be considered of great interest with respect to $\beta$-glucosidase, which is meant to break such natural glycosides. The presented work comprises the enzymatic functionality exploration and thus it becomes highly important to emphasise the enzymatic synthesis glycosides.

There are two classes of biocatalysts available for the enzymatic synthesis of glycosides: the glycosidases or transglycosidases and the glycosyltransferases. Glycosidases have the advantage that they are often readily available and use simple glycosyl donors. Because glycosidases usually catalyse the reverse reaction (hydrolysis), reaction conditions need to be carefully chosen to encourage synthesis, for example by using a large excess of acceptor and an activated donor. Glycosidases are generally not as regioselective as transferases but, with the correct combination of enzyme and substrate, single isomers can be formed. A recent example is the synthesis of the $\alpha$-galactosyl epitope, which was synthesized from N-acetyllactosamine and $\alpha$-$p$-nitrophenylgalactoside in 48% yield. A further improvement of the method is the conversion of glycosidases to glycosynthases by sitedirected mutagenesis. The application of method developed by Fort et al. (2000) yielded a highly efficient mutant cellulase that was used for $\beta$-1-4-linked oligosaccharides and polysaccharides. An interesting modification of reaction conditions to drive the glycosylation reaction forward proposed by Matsumura et al. (1999) stated the use of supercritical carbon
dioxide as a solvent, which has been applied to the synthesis of octyl-
xylobiosides and xylotriosides.

Glycosyltransferases are used in nature to catalyse the formation of
glycosidic bonds in high yields with excellent selectivity. As catalytic agents, they
tend to be less readily available and more expensive than the glycosidases; however, they are more useful for glycosidic linkages that are difficult to make
by other means such as the β-mannosidic linkage or α-sialosides (Koeller and
Wong, 2000). Glycosyltransferases are also very useful for the efficient synthesis
of larger oligosaccharides and glycoconjugates from chemically synthesised
building blocks. Two recent examples are the chemoenzymatic synthesis of 7
(Koeller and Wong, 2000) and 8 (Depre et al., 1999) using fucosyl-
and sialyltransferases in the last step of the synthesis after final deprotection of the
chemically synthesised core saccharides. The macromolecular nature of their
natural substrates makes glycosyltransferases also ideal enzymes for
oligosaccharide synthesis on the solid phase (Sallas and Nishimura, 2000). The
repertoire of reactions that can be catalyzed by glycosyltransferases and
glycosidases is ever increasing, spurred on by their biological relevance and their
use in in vitro synthesis; therefore, more and more enzymes are being isolated
and over expressed heterologously. Seto et al. (2000) aided the applications of
mutagenesis methods on naturally occurring transferases to modify their
substrate specificity.

2.4. β-Glucosidase

β-Glucosidase (β-D-glucoside glucohydrolases; EC 3.2.1.21) catalyzes
hydrolysis of a wide range of β-glucosides including alkyl-, aryl-, β-glucosides,
diglucosides, oligosaccharides etc. In general, glucosidases encompass a
heterogeneous group of enzymes cleaving β 1-4 linkages of di- and oligo-
saccharides, or other gluco conjugates with hetero molecules (aglycones) and
occurs ubiquitously in eukaryotes, eubacteria and archaea (Woodward and
β-Glucosidases have been classified according to various criteria and thus there is no single well-defined method for the classification of these versatile enzymes. Henrissat (1996) exposed two bases for the classification of β-glucosidases, (i) substrate specificity, and (ii) nucleotide sequence identity. One of the first classification schemes based on the available sequences proposed grouping of these enzymes into two types, namely, Type I and Type II β-glucosidases. Another proposed scheme divided β-glucosidases into two subfamilies, subfamily A (BGA) and subfamily B (BGB). However, a classification based on substrate specificity states about -aryl-β-glucosidases, -true cellobiosiases, and -broad substrate specificity enzymes classes which are also active on a wide array of substrates. These enzymes display broad substrate specificity with respect to the aglycone portion of the substrate. Numerous studies have provided insight into the substrate specificity of β-glucosidases isolated from different organisms wherein, most of the family 1 enzymes show significant β-galactosidases activity. β-Glycosidases have been classified into one of the 57 families of glycosidases, and β-glucosidases are classified as Family 1 glycosidases based on amino acid sequence similarity and substrate specificity. A remarkable feature of the enzymes belonging to this family is a wide range of substrate preferences despite high sequence homology (Henrissat and Romeus, 1995; Henrissat, 1996).

In brief, the classification scheme proposed for all glycosylhydrolases (nearly 2000 in number) resulted in recognition of 88 families. β-Glucosidases with the exception of glucosylceramidase (acid β-glucosidase), a member of family of 30, are placed in either family 1 or family 3 of glycosylhydrolases. Family 1 comprises nearly 62 β-glucosidases from archaeabacteria, plants, mammals, and also includes 6-phosphoglycosidases and thioglycosidases. Most family 1 enzymes also show significant β-galactosidase activity. The family 1 β-glucosidases are also classified as members of the 4/7 super family with a
common eight-fold $\beta/\alpha$ barrel motif. Here, the active site is placed in a wide cavity defined along the axis of the barrel, with a putative acid/base catalyst located at the end of $\beta$-strand 4 and a catalytic nucleophile near the end of $\beta$-strand 7. The 4/7 super family also includes other enzymes like family 2 $\beta$-galactosidase, family 5 cellulases, family 10 xylanase, and family 17 barley glucanases. Family 3 of glycosylhydrolases consists of nearly 44 $\beta$-glucosidases and hexosaminidases of bacterial, mold, and yeast origin (Bhatia et al., 2002).

2.4.1. $\beta$-Glucosidases from microbes

Microbial $\beta$-glucosidases have been the subject of extensive research and have given a lot of promising information regarding the functions and characters of the enzyme. The cellulolytic enzymes are composed of three main activities: endoglucanase, exoglucanase and $\beta$-glucosidase, are wide spread among bacterial and fungal strains even though the exoglucanases (also called cellobiohydrolases) are rare in the bacterial kingdom. More than hundred of endo and exoglucanase sequences are known. Cellulolytic enzymes are also known to play an important role in some industrial applications, such as in bio-stone washing of jeans, replacing the stones in their abrasive effect on the garments. $\beta$-Glucosidases are also important in the regulation of cellulase genes since they are the key enzyme in the synthesis of sophorose, an efficient inducer of the cellulolytic system of *Trichoderma reesei* (Bhatia et al., 2002). They constitute also the focus of many applied researches since they are not only needed in the cellulose breakdown but also in the synthesis of oligomers and other complex molecules (such as alkyl-glucosides) by transglycosylation (Bhatia et al., 2002).

One of the earliest bacterial $\beta$-glucosidase, purified from *Agrobacterium faecalis* by Day and Withers (1986) was a dimer of 50 kDa monomer and exhibited high specificity for cellobiose (Trimbur et al., 1992). The fungal enzymes are used in several biotechnological processes, including development of novel carbohydrate foods, alcohol based fuels and other commercial products from
cellulose. Particularly, glucose production can be achieved from the most abundant biological macromolecule, cellulose, by the extracellular enzyme complex (cellulase) that is derived from various fungal species such as *Trichoderma*. A cellulase complex isolated by Fowler (1993) from *Trichoderma reesei* comprised at least three different enzymes that together hydrolyze cellulose to oligosaccharides and glucose. Of these, the endoglucanases and cellobiohydrolases synergistically hydrolyze cellulose into small cellooligosaccharides, mainly cellobiose.

Another major application of microbial β-glucosidases is in the flavor and fragrance industry. Gueguen *et al.* (1996) reported that β-glucosidases are key enzymes in the release of aromatic compounds from glucosidic precursors present in fruits and fermenting products. Indeed, many natural flavor compounds, such as monoterpenols, C-13 norisoprenoids, and shikimate-derived four compounds, accumulate in fruits as flavorless precursors linked to mono- or diglycosides and require liberation by enzymatic or acidic hydrolysis (Vasserot *et al.*, 1995; Winterhalter and Skouroumonis, 1997). Microbial β-glucosidases can also be used to improve the organoleptic properties of citrus fruit juices, in which the bitterness is in part due to a glucosidic compound. The studies by Gunata *et al.* (1985) showed that monoterpenols in grapes (e.g., linalol, geraniol, nerol, citronelol, α-terpineol and linalol oxide) are linked to diglucosides, which contribute to the flavor of wine. The enzymatic hydrolysis of these compounds requires a sequential reaction, which produce monogluco sides. Subsequently, monogluco sides are hydrolyzed by the action of β-glucosidases. Endogeneous β-glucosidases from grape are not sufficient to process the hydrolysis of monoterpenyl-glucosides. The grape enzymes display limited activity towards these glucosides and a large fraction of the aromatic compounds remains unprocessed in mature fruit. The addition of glucose-tolerant exogenous β-glucosidase isolated from fungi (*Aspergillus oryzae*) was shown to improve the
hydrolysis of glucoconjugated aromatic compounds and enhance wine quality (Riou et al., 1998).

Enzymes derived from thermophilic microorganisms are often preferred for the transformation of lactose in milk or whey because of their various advantages such as increased thermostability, reduction in growth of unwanted microbial contaminants at elevated temperatures, and reduction in viscosity of the reaction system. An intracellular β-glycoside hydrolase with β-glucosidase and β-galactosidase activity was isolated by Nakkharat and Haltrich (2006) from the thermophilic ascomycete Talaromyces thermophilus. Amouri and Gargouri (2006) reported an improved mutant cellulolytic fungal (Stachybotrys sp.) β-glucosidase which showed activity towards salicin, methyl-umbellypheryl-β-S-glucoside and p-nitrophenyl-β-D-glucoside, thus it showed a true β-glucosidase activity since it splits cellobiose into two glucose monomers. The enzyme showed more affinity to pNPG than cellobiose and salicin. Another inducible mycelial β-glucosidase from Scytalidium thermophilum reported by Zanoelo et al. (2004) showed wide sustrate specificity and was activated by glucose or xylose which is a distinguished character of this enzyme from all others. Like Scytalidium, an extracellular β-glucosidase from wooddecaying fungus Daldinia eschscholzii showed p-NP-β-D-glucopyranoside specificity and was competitively inhibited by glucose. The internal amino acid sequences of D. eschscholzii β-glucosidase have similarity to the sequences of the family 3 β-glucosyl hydrolase (Karnchanatat et al., 2007).

Microbial β-glucosidases are vastly implicated in biotechnological application and have been well-characterized. Many of microbial β-glucosidases are reported to their physico-kinetic level characterization which will be used to highlight the present comparative study.
2.4.2. β-Glucosidases from animals

In animals especially humans, several β-glucosidases have been described for their physiological role in metabolism. For example, the lysosomal β-glucosidase (acid β-glucosidase) hydrolyzes glucocerebrosides (glycosphingolipids) present in the lysosomal membranes, and a lack of this enzyme is the cause of the various forms of Gaucher's disease, one of the hereditary lysosomal storage disorders. A membrane associated glucocerobrosidase (EC 3.2.1.45) reported by Beutler (1992) was weakly glycosylated enzyme in lysosome which cleaved the substrate glucosylceramide (glucocerebroside) to ceramide and glucose. Lysosomal β-glucosidase is present in most tissues and cell types with various levels of catalytic activity. A deficiency of the lysosomal β-glucosidase in humans results in a condition known as Gaucher disease. Seven different mutations in human β-glucosidase have shown to result in inactive enzymes (Ohashi et al., 1992).

Cytosolic β-glucosidases are mainly present in the liver, kidney and intestine and are reported to play a key role in detoxification of plant β-glucosides which was based on the broad specificity towards aglycone moieties of mono and disaccharide substrates such as L-picein, salicin, arbutin, amygdalin, prunasin, visine and linamarin that are found in plants consumed by animals. The study on first heterologous expression of human CBG was accomplished by Berrin et al. (2002). A full-length CBG cDNA (cbg-1) was cloned from a human liver cDNA library and expressed in the methylotrophic yeast Pichia pastoris. It has been reported that the human enzyme had significant activity towards many common dietary xenobiotics including glycosides of phytoestrogens, flavonoids, simple phenolics and cyanogens with higher apparent affinities for dietary xenobiotics than for other aryl-glycosides. These data indicated that human CBG hydrolyzed a broad range of dietary glucosides and may play a critical role in xenobiotic metabolism (Berrin et al., 2002). In a recent study, Tribolo et al. (2007) advocated that human cytosolic β-glucosidase (hCBG) was a xenobiotic-
metabolizing enzyme that hydrolyzes certain flavonoid glucosides, with specificity depending on the aglycone moiety, the type of sugar and the linkage between them.

A putative protein, predicted from the klotho (kl) gene showed homology to family 1 glycosyl hydrolase and was also predicted to occur in the cytosol of certain human cells where it might have a role in human aging (Kuro et al., 1997). Another human β-glucosidase was specific for the hydrolysis of pyridoxine 5′-β-D-glucopyranoside, a common dietary form of vitamin B6, and has been ascribed a role in vitamin B6 bioavailability (McMahon et al., 1997).

A β-glucosidase with broad regiospecific activity from China white jade snail (Achatina fulica) was found to cleave both β-(1→2)-glucosidic linkage at 3-C and β-(1→6)-glucosidic linkage at 20-C of ginsenosides and can hydrolyze ginsenosides Rb1, Rb2, Rb3 and Rc. The enzyme specifically hydrolyzed the β-D-glucosides involving aryl-β-glucosides, alkyl-β-glucosides, and β-linked disaccharides (i.e. sophorose, gentiobiose, and cellobiose) (Hu et al., 2007). Insecta have shown interesting β-glucosidase system; a β-glucosidases from the ventriculus and honey sac and the hypopharyngeal glands of Apis mellifera reported by Pontoh and Low (2002) were glycoprotein.

Abstracting from detailed studies on animal β-glucosidase protein and nucleic acid researches it appears that the β-glucosidase will provide the basis for future studies on the physiological role of this enzyme.

2.4.3. β-Glucosidases from plant kingdom

β-Glucosidase has occupied a variety of roles and has been well characterized for its significant metabolic functionality in plants biological processes and potential biotechnological applications. It plays an important role in defense against some pathogens and herbivores by releasing hydroxamic acids, coumarins, thiocyanates, terpenes, and cyanide from the corresponding glucosides (Fenwick et al., 1983; Jones, 1988; Niemeyer, 1988; Poulton, 1990;
Plant β-glucosidases also function in the hydrolysis of conjugated phytohormones (i.e., glucosides of gibberellins, auxins, abscisic acid, and cytokinins) (Wiese and Grambow, 1986; Brzobohaty et al., 1993). One of the finest implicit functions of β-glucosidases in plants is the hydrolysis of the cyanogenic glucosides (cyanogenesis). In this case, the enzyme and substrate occur in different subcellular compartments and come into contact with each other only after tissue disruption by pathogens or herbivores. As a result, the cyanogenic glucosides are hydrolyzed, releasing toxic hydrogen cyanide (HCN). Many economically-important crops, including sorghum, cassava, lima beans and cherries accumulate cyanogenic glucosides and the hydrolysis of these compounds produces the respiratory poison HCN (Conn, 1981; Jones, 1988; Poulton, 1988; Oxtoby et al., 1991). The studies on white clover (Trifolium repens L) by Hughes (1993) revealed two related cyanogenic glucosides (linamarin and lotaustralin) were the substrates of linamarase (β-glucosidase), which is a glycosylated protein having high-mannose-type N-asparagin-linked oligosaccharides which hydrolyze to glucose and HCN. Linamarases are localized the cell walls of the epidermal cells of leaves (Kakes, 1985; 1993). Other cyanogenic linamarase from cassava has been studied in detail by various researchers (Mkpong et al., 1990; Yeoh and Woo, 1992; Hughes, 1993). The cassava plant is a staple food in most African countries which is a highly cyanogenic plant and causes acute cyanide poisoning in humans (Poulton, 1989). Hosel et al. (1987) have reported two dhurrinases (Dhurrinase 1 and Dhurrinase 2) from Sorghum seedling which shared a number of characteristics feature with maize and plastid localization (Esen and Stetler, 1993).

Bennett et al. (1997) reported a different type, dicot β-glucosidase (myrosinase; thioglucoside glucohydrolase, EC 3.2.3.1)) from family Brassicaceae catalyzed the hydrolysis of secondary compounds, glucosinolates (thioglucosides), which contained a glucose residue with a sulfur- and nitrogen-containing side chain. In oilseed rape three structural classes of glucosinolates,
derived from phenylalanine (aromatic), methionine (aliphatic/alkenyl) and tryptophan (indolyl) were reported by Fenwick et al. (1983). Glucosinolates are stored in the vacuoles of plants and when the tissue is disrupted they are converted by myrosinase to toxic compounds such as isothiocyanates, thiocyanates, and nitriles. The resulting byproducts of the reaction cause thyroid and liver disease in animals and humans who consume glucosinolate-rich foods such as rape (Brassica napus). This enzyme-substrate system is implicated as a defense system against herbivorous insects, slugs, and fungal pathogens as well as being involved in host-plant recognition by specialized insect pests of oilseed rape (Fenwick et al., 1983). Falk and Rask (1999) reported a β-glucosidase from seeds of Brassica napus (oilseed rape) had 130 kDa native molecular mass consisted of a disulfide linked dimer of 64 kDa monomers and the internal amino acid sequences were used in cloning of cDNA for the enzyme. One nearly full-length and one partial β-glucosidase-encoding cDNA clone were isolated and sequenced. Zeatin-O-glucoside was identified as a natural substrate for B. napus β-glucosidase.

A detailed study on maize (Zea mays L.) β-glucosidase has been carried out by Esen (1992). Cuevas et al. (1992) reported that maize β-glucosidase cleaves hydroxamic acid glucosides (HxGlc), particularly 2-O-β-D-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOAGlc). The aglycone DIMBOA is present as a glucoconjugate (DIMBOAGlc) in intact tissues of maize, rye and wheat. DIMBOA is a potent, toxic aglycone that has been shown to inhibit the electron transport system and phosphorylation reactions in bovine mitochondria and spinach chloroplasts (Niemeyer et al., 1986).

Another plasticidal β-glucosidase from oat (Avena sativa) reported by Gus-Mayer et al. (1994) was found to be involved in the defense mechanism against fungal infection, destruction of the oat cell wall and plasma membrane, elicited by fungal infection, brings the plastidal β-glucosidase in contact with the vacuolar avenacosides. Consequently, the enzyme hydrolyzed inactive substrates
into active 26-desglucoavenacosides, which has been shown to possess antifungal activity. Based on the nucleotide sequence, the oat \( \beta \)-glucosidase is classified as a member of the family 1 glycosyl hydrolase (Gus-Mayer et al., 1994). Minami et al. (1999) isolated a plasticidal \( \beta \)-glucosidase from leaves of the indigo plant (*Polygonum tinctorium*) which contained high activity for the substrate indican.

A germinating rice seed \( \beta \)-glucosidase ionically bound to cell walls was purified by Akiyama et al. (1998) and shown to have an apparent Mr 56 kDa with its N-terminal amino acid sequence (44 residues) exhibiting a high homology with those of \( \beta \)-glucosidases from other plants, such as barley and white clover. The enzyme showed hydrolytic as well as transglycosylation activity towards \((1\rightarrow3)\)-\( \beta \)- and \((1\rightarrow4)\)-\( \beta \)-linked oligosaccharides. This suggested that the \( \beta \)-glucosidase is probably involved not only in hydrolysis but also in modification of oligosaccharides in cell walls of germinating rice seeds (Akiyama et al., 1998). In another study Opassiri et al. (2003) reported that the cDNAs for two \( \beta \)-glucosidase isozymes from rice (*Oryza sativa* L.), designated *hglu1* and *hglu2* were cloned and sequenced which included open reading frames encoding 504 and 500 amino acid precursor proteins, respectively. However, differences were seen in expression in mature plants, where *hglu1* was highly expressed in flowers, but *hglu2* was not.

A \( \beta \)-glucosidase from rye (*Secale cereale*) shoots reported by Sue et al. (2000) was highly active not only on DIMBOA-Glc but also on its 7-demethoxy analogue as substrate, DIBOA-Glc-, a substrate specificity different from those of maize and wheat glucosidases. The wheat (*Triticum aestivum*) and rye (*Secale cereale*) \( \beta \)-D-glucosidases have been shown to hydrolyze hydroxamic acid-glucose conjugates and one distinctive property of the wheat and rye glucosidases is that they function as hexamers but lose activity when dissociated into smaller oligomers or monomers (Sue et al., 2006). Similarly, a glycosidase containing \( \beta \)-glucosidase and \( \beta \)-fucosidase activities from *Dalbergia cochinsinensis* Pierre (Thai
Rosewood) has been reported by Srisomsap et al. (1996) to be 66 kDa pentahomodimeric subunit structure of 330 kDa native protein.

There are more than 120,000 secondary metabolites known to occur in higher plants and many of them are important because of various pharmacological and therapeutic applications (Gerasimenko et al., 2002). Warzecha et al. (1999) studied the plant cell suspension cultures of Rauwolfia which contained a 61 kDa raucaffricine-O-β-D-glucosidase was found to be a new member of the family 1 of glycosyl hydrolases. Plants of Rauwolfia serpentina accumulate ajmaline as a major alkaloid, whereas cell suspension cultures of Rauwolfia mainly accumulate the glucoalkaloid raucaffricine. Cell cultures do contain a specific glucosidase known as raucaffricine-O-β-D-glucosidase (RG), which catalyzes the in vitro formation of vomilenine, a direct intermediate in ajmaline biosynthesis. Zárate et al. (2001) established different transgenic cell lines of Nicotiana tabacum expressing strictosidine β-glucosidase cDNA from Catharanthus roseus following Agrobacterium tumefaciens infection. Molecular data showed that C. roseus cells SGD activity was associated with a protein aggregate of a size of 650 kDa, and this was absent in control and samples of the transgenic lines which failed to show SGD activity (Zárate et al., 2001).

Among several fruit seeds, apple seed was identified as a new promising β-glucosidase source for alkyl O-glucoside synthesis by reverse hydrolysis, since it showed high hydrolytic activities on a broad spectrum of β-glucosides. From the apple seed meal, a major glucosidase isoenzyme reported by Yu et al. (2007) showed higher thermal stability than β-glucosidase from almond preserved at 50 °C in an aqueous environment.

Winemaking is a biotechnological process in which the use of exogenous enzyme preparations helps to overcome the problem of the insufficient activity of endogenous activity in the grapes. The use of the enzymes in the wine industry remains limited for several reasons viz. traditionalism of winemakers, influence on enzymatic activities related to physicochemical characteristics of musts and
wines (pH, temperature, ethanol, sugars, polyphenols, etc.) on enzymatic activities. The major enzyme groups in winemaking are oxidoreductase, pectinase, protease and finally β-glucosidase. Any study of β-glucosidase enzymes must address the terpene compounds that contribute to the varietal character of wines. Reports indicate that not all glycosides are present in all grape varieties, and that concentrations vary according to variety (Gunata et al., 1985). Major precursors include structures such as β-D-glucopyranoside, 6-O-α-L-arabinosyl-β-D-glucopyranoside, 6-O-α-L-rhamnopyranosyl-β-D-glucopyranoside and 6-O-β-D-apiofuranosyl-β-D-glucopyranoside apiosylglycosides. Studies by Villena et al. (2007) confirmed that aglycon chemical structure (volatile when free) may vary, taking the form of a terpenol (linalol, geraniol, nerol, citronelol, hortienol or α-terpinol), linalol oxide, linear or cyclic alcohol (hexanol, phenylethanol, benzyl alcohol), C13 norisoprenoid, phenolic acid and/or volatile phenol.

Dignum et al. (2004) reported a β-glucosidase from green vanilla (Vanilla planifolia) beans which showed activity towards eight naturally occurring glucosides in vanilla and towards p-nitrophenol. The enzyme does not have high substrate specificity for the naturally occurring glucosides compared to the synthetic p-nitrophenol glucoside. A high specificity β-glucosidase active on isoflavone conjugates from soybean (Glycine max) roots was highly activity against isoflavone conjugates. The enzyme was inactive against tested flavonol glycosides and it was supposed that this enzyme is involved in the release of daidzein and genistein, both of which play central roles in soybean defense (Hsieh and Graham, 2001). Anthocyanin content in Sicilian sweet orange (Citrus sinensis (L) Osbeck) varieties known as blood oranges (Tarocco, Moro sanguinello) undergoes changes during the ripening process. The anthocyanin concentration reaches to maximum in the fully ripe fruit. At latter stage of maturity, a decrease of these pigments is observed. β-Glucosidase activity in Tarocco variety, the most
common Sicilian blood orange was estimated in order to underline its role on anthocyanins degradation during ripening (Barbagallo et al., 2007).

Thus, β-glucosidase occurs ubiquitously in living organisms belonging to all kingdoms but performs varied functions in them. Plant β-glucosidases are known to function in chemical defense of young plant parts against pests by catalyzing the hydrolysis of toxic glucosides. β-Glucosidases accounts for the liberation of the flavoring compounds collectively called terpenols (nerol, geraniol, linalool), benzyl and phenylethyl alcohols from their respective glucoside precursors. Additionally, its functions in plants include the hydrolysis of phytohormone precursors, pigment metabolism, seed development, and biomass conversion.

2.4.4. Substrate specificity of β-glucosidases

β-Glucosidases exhibit broad specificity with respect to both the aglycone and the glycone moieties of their substrates. In fact, true β-glucosidases from all sources have a similar specificity for the glycone (glucose) portion of the glucoside; however they vary with respect to aglycone specificity. The cyanogenic diglycoside (R)-amygdalin (the gentiobioside of (R)-mandelonitrile) that accumulates in black cherry seeds and other stone fruits contains a disaccharide as the glycone part of the substrate (Poulton, 1993). Studies by Svasti et al. (1999) on an isoflavonoid glucoside from the seeds of Thai Rosewood (Dalbergia cochinchinensis Pierre) showed high specificity to 12-dihydroamorphigenin, dalcochinin. Dalcochinin-8′-O-β-D-glucoside is hydrolyzed by Thai Rosewood β-glucosidase, but not by other β-glucosidases, such as cassava linamarase, almond β-glucosidase, or mustard seed myrosinase. Similarly, a β-glucosidase from Vanilla planifolia reported by Dignum et al. (2004) was found to be involved in aroma generation expressed its highest affinity for a glucoside with a polar group in the para-position (p-nitrophenol, vanillin, vanillic acid, p-hydroxybenzaldehyde, and ferulic acid). Glucosides with an apolar or no
substituent in the \textit{para}-position and a methoxyl-group in \textit{ortho}-position are hydrolyzed poorly (guaiacol and creosol). Compounds lacking a polar group in the \textit{para}-position and a methoxyl-group in \textit{ortho}-position (\textit{p}-cresol and 2-phenylethanol) or that have the glucose attached to the sidechain (2-phenylethanol) are not hydrolyzed at all.

Srisomsap \textit{et al.} (1996) exposed an enzyme containing \textit{\beta}-glucosidase and \textit{\beta}-fucosidase activity from \textit{Dalbergia cochinchinensis} Pierre which showed high efficacy towards \textit{p}-NP-\textit{\beta}-D-glucoside and a slow hydrolysis of \textit{p}-NP-\textit{\beta}-D-galactoside, \textit{p}-NP-\textit{\beta}-D-xyloside, and \textit{p}-NP-\textit{\alpha}-L-arabinoside. Hydrolysis of sophorose, laminaribiose, and gentiobiose were slow and cellobiose hydrolysis was slowest reported. Cynogenic glucoside linamarin or prunasin were not hydrolyzed, but a little hydrolysis of amygdalin and salicin was recorded. Another \textit{\beta}-glycosidase from the seeds of \textit{Dalbergia nigescens} Kurz was reported by Chuankhayan \textit{et al.} (2005) which showed its ability to hydrolyze \textit{p}-nitrophenyl-\textit{\beta}-D-glucoside and \textit{\beta}-fucoside but this enzyme did not hydrolyze various glycosidic substrates efficiently and thus it was used to identify its own natural substrates. Two substrates were identified, isolated and their structures determined as: dalpatein \textit{7-O-\beta}-D-apiofuranosyl-(1→6)-\textit{\beta}-D-glucopyranoside and \textit{6,2',4',5'-tetramethoxy-7-hydroxy-7-O-\beta}-D-apiofuranosyl-(1→6)-\textit{\beta}-D-glucopyranoside (dalnigrein \textit{7-O-\beta}-D-apiofuranosyl-(1→6)-\textit{\beta}-D-glucopyranoside). The \textit{\beta}-glycosidase removes the sugar from these glycosides as a disaccharide, despite its initial identification as a \textit{\beta}-glucosidase and \textit{\beta}-fucosidase. \textit{\beta}-D-Glucan glucohydrolases can hydrolyze glycosidic linkages in several \textit{\beta}-D--glucans, in \textit{\beta}-D-oligoglucosides containing (1→2)-, (1→3)-, (1→4)-, or (1→6)-linkages, in aryl \textit{\beta}-D-glucosides such as \textit{p}-nitrophenyl-\textit{\beta}-D-glucopyranoside, and in some \textit{\beta}-D-oligoxylglucosides. The barley \textit{\beta}-D-glucan glucohydrolases reported by Hrmova \textit{et al.} (1998) was also found to hydrolyze cyanogenic \textit{\beta}-D-glucosides but with low activity. Their broad substrate specificity makes it difficult to assign these higher plant \textit{\beta}-D-glucan glucohydrolases to current Enzyme Commission
classes; therefore, they have been described variously as β-D-glucan glucohydrolases, (1→3)-β-D-glucan exohydrolases, and β-D-glucosidases (Hrmova et al., 1998; Varghese et al., 1999).

Family 3 β-D-glucan glucohydrolases catalyze the hydrolytic removal of β-D-glucosyl residues from nonreducing termini of a range of β-D-glucans and β-D-oligoglucosides. Their broad specificity was explained by Hrmova et al. (2002) using x-ray crystallographic data obtained from a barley β-D-glucan glucohydrolase in complex with nonhydrolyzable S-glycoside substrate analogs and by molecular modeling of enzyme/substrate complexes. The glucosyl residue that occupies binding subsite -1 was locked tightly into a fixed position through extensive hydrogen bonding with six amino acid residues near the bottom of an active site pocket. In contrast, the glucosyl residue at subsite -1 was located between two Trp residues at the entrance of the pocket, where it is constrained less tightly. The relative flexibility of binding at subsite -1, coupled with the projection of the remainder of bound substrate away from the enzyme’s surface, means that the overall active site can accommodate a range of substrates with variable spatial dispositions of adjacent β-D-glucosyl residues. The broad specificity for glycosidic linkage type enables the enzyme to perform diverse functions during plant development (Hrmova et al., 2002).

Among glycoside hydrolases, β-glucosidase plays a unique role in many physiological and biocatalytical processes that involve the β-linked O-glycosyl bond of various oligomeric saccharides or glycosides. Structurally, the enzyme can be grouped into glycoside hydrolase family 1 and 3. Although the basic (“retaining, double-displacement”) mechanism for the catalysis of family 3 β-glucosidase has been established, in-depth understanding of its structure–function relationship, particularly the substrate specificity that is of great interest for developing the enzyme as a versatile biocatalyst, remains limited. To further probe the active site, a comparative study was performed by Langston et al. (2006) on a family 3 β-glucosidase from Aspergillus oryzae with substrates and
competitive inhibitors of different structures, in attempt to evaluate the site-specific spatial and chemical interactions between a pyranosyl substrate and the enzyme. Plant β-glucosidases display varying substrate specificities; Verdoucq et al. (2003) reported that the maize β-glucosidase isozyyme Glu1 (ZmGlu1) was found to hydrolyze a broad spectrum of substrates in addition to its natural substrate DIMBOA-Glc (2-O-β-D-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoaxin-3-one), whereas the sorghum β-glucosidase isozyyme Dhr1 (SbDhr1) hydrolyzed exclusively its natural substrate dhurrin (p-hydroxy-(S)-mandelonitrile-β-D-glucoside). Structural data of enzyme-substrate and enzyme-aglycone complexes have showed that five amino acid residues (Phe198, Phe205, Trp279, Phe466, and Ala467) are located in the aglycone-binding site of ZmGlu1 and form the basis of aglycone recognition and binding, hence substrate specificity. Verdoucq et al. (2003) studied the mechanism of substrate specificity further, mutant β-glucosidases were generated by replacing Phe198, Phe205, Asp261, Met263, Phe277, Phe466, Ala467, and Phe473 of Glu1 by Dhr1 counterparts. The simple mutant replacing Phe198 by a valine had the most drastic effect on activity, because the capacity of this enzyme to hydrolyze β-glucosides was almost completely abolished. The analysis of this mutation was completed by a structural study of the double mutant ZmGlu1-E191D, F198V in complex with the natural substrate. The structure reveals that the single mutation F198V caused a cascade of conformational changes, which were unpredictable by standard molecular modeling techniques. Some other mutations led to drastic effects: replacing Asp261 by an asparagine decreases the catalytic efficiency of this simple mutant by 75% although replacing Tyr473 by a phenylalanine increased its efficiency by 300% and also provided a new substrate specificity by hydrolyzing dhurrin (Verdoucq et al., 2003).
2.4.5 Transglycosylation by glycosidases

β-Glucosidases normally catalyze the hydrolysis of β-glucosidic linkages between D-glucose and an aglycone or another sugar and are evolutionarily related. Like other glycosidases, some β-glucosidases may catalyze reverse hydrolysis and transglucosylation, leading to synthesis of oligosaccharides and alkyl glucosides. Particularly, in the presence of alcohols, alkyl glucosides may be formed by reverse hydrolysis using glucose or by transglucosylation using sugar donors such as p-NP-β-D-glucopyranoside or cellobiose. Under certain conditions, the reverse of hydrolysis, that is, synthesis of glycosyl-bond between different molecules can occur. This takes place via two different modes, reverse hydrolysis and transglycosylation. In the first approach, modification of reaction conditions such as lowering of water activity (a_w), trapping of product or high substrate concentration leads to a shift in the equilibrium of reaction toward synthesis. This reaction is under thermodynamic control. In transglycosylation approach, a preformed donor glycoside (e.g., a disaccharide or aryl-linked glucoside) is first hydrolyzed by the enzyme with the formation of an enzyme-glycosyl intermediate. This is then trapped by a nucleophile other than water (such as a monosaccharide, disaccharide, aryl-, amino-, or alkyl-alcohol or monoterpene alcohol) to yield a new elongated product (Bhatia et al., 2002).

Synthesis of alkyl glucosides has mainly employed high catalytically efficient almond β-glucosidase as well as Fusarium oxysporum and Pyrococcus furiosus β-glucosidases (Svasti et al., 2003). Srisomsap et al. (1996) reported a β-glucosidase (dalcochinase) from the seeds of Thai rosewood (Dalbergia cochinchinensis Pierre) which catalyzed reverse hydrolysis using glucose as substrate, yielding di- and tri-saccharides and can also transfer glucose from p-NP-β-D-glc to alkyl alcohols. However, like almond and Fusarium β-glucosidases, dalcochinase uses primary alcohols as acceptors better than secondary alcohols, and cannot use tertiary alcohols at all. The inability of β-glucosidases to synthesize tertiary alkyl glucosides by transglucosylation or by reverse
hydrolysis appears to be a general phenomenon found with all glycosidases. In a report, Svasti et al. (2003) explored the exceptional ability of the cyanogenic β-glucosidase (linamarase) from cassava (Manihot esculenta Crantz) to transfer glucose to tertiary alcohols, and compare its action to that of Thai rosewood and almond β-glucosidases. These were reported to synthesize alkyl glucosides by transglucosylating alkyl alcohols of chain length C1–C8. Cassava linamarase shows greater ability to transfer glucose from p-nitrophenyl-β-glucoside to secondary alcohol acceptors than other β-glucosidases, and is unique in being able to synthesize C4, C5, and C6 tertiary alkyl β-glucosides with high yields of 94%, 82%, and 56%, respectively. Cassava linamarase required p-NP-glycosides as donors and could not use mono- or di-saccharides as sugar donors in alkyl glucoside synthesis (Svasti et al., 2003).

Hansson et al. (2001) evaluated five different β-glycosidases (Pyrococcus furiosus β-glucosidase, Sulfolobus solfataricus β-galactosidase, Caldocellum saccharolyticum β-glucosidase, almond β-glucosidase and Escherichia coli β-galactosidase) as transglycosylation catalysts in hexanol containing various amounts of water. All enzymes catalyzed both hydrolysis and transglycosylation of the glycosidic substrates (pentyln- and p-nitrophenyl-β-D-glucoside and p-nitrophenyl-β-D-galactoside). From the concentration ratio (alcohol/water) a decrease in ratio of the transglycosylation tohydrolysis ratio was expected with increasing water activity in the hexanol. However, for all enzymes tested the selectivity for the alcohol increased with increasing water activity. This counteracted the effect of higher water concentration and in most cases the transglycosylation/hydrolysis ratio increased with increasing water activity. On the other hand, in hexanol/water two-phase systems, hydrolysis has been shown to be by far dominating reaction even though the total activity increased for all enzymes. However, Hansson et al. (2001) found deviations in cases when the reactions became thermodynamically controlled: at high water contents secondary hydrolysis reduced the transglycosylation yields while higher
transglycosylation yields than predicted were obtained at low water activity in some cases using enzymes poorly selective for the alcohol.

In a detailed study, Hays et al. (1998) elaborated that cytosolic β-glucosidase (CBG) catalyzes a variety of transglycosylation reactions, which have been shown with other glycosylhydrolases to function in synthetic and genetic regulatory pathways. The catalytic mechanism, substrate specificity, and transglycosylation acceptor specificity of guinea pig liver CBG was investigated by several methods. These studies indicated that CBG employed a two-step catalytic mechanism with the formation of a covalent enzyme-sugar intermediate and that CBG will transfer sugar residues to primary hydroxyls and equatorial but not axial C-4 hydroxyls of aldopyranosyl sugars. Further analyses revealed that for aldopyranosyl substrates, the activation energy barrier is affected most by the presence of a C-6 carbon and by the configuration of the C-2 hydroxyl, whereas the binding energy is affected modestly by the configuration and substituents at C-2, C-4, and C-5. These indicated that the transglycosylation activity of CBG derived from the formation of a covalently linked enzyme-sugar intermediate and that the specificity of CBG for transglycosylation reactions is different from its specificity for hydrolysis reactions. The enzyme hydrolyzed β-D-galactopyranosides, β-D-fucopyranosides, β-D-xylopyranosides, and α-L-arabinopyranosides, in addition to β-D-glucopyranosides. The enzyme also catalyzed transglycosylation reactions in which a sugar residue is transferred from a substrate molecule to an acceptor to form a new glycoside. These properties are consistent with the fact that CBG is a configuration-retaining glycosidase. Collectively, Hays et al. (1998) suggested that the catalytic mechanism of CBG consists of a double-displacement reaction involving the formation of a stable enzyme-sugar intermediate.

Transglucosylation activity of rice (*Oryza sativa* L.) βGlu1 has been ascertained by Opasiri et al. (2004) using its ability to transfer glucose from pNPG to pyridoxine (vitamin B6). There were five major transglucosylation
products, as expected, four of them were pNP derivatives with the same Rt values as products of the control containing only enzyme and pNPG. These transfer products were compared that one product had an Rt value equal to that of pNP-β-D-cellobioside, and another had an Rt equal to that of pNP-β-D-cellotrioside. After hydrolysis transglucosylation product was identified to be pyridoxine 5′-O-β-D-glucopyranoside (Opassiri et al., 2004).

A high catalytic active almond β-glucosidase has been used in transglycosylation reactivity and the stability of almond β-glucosidase in five different organic media was evaluated by Kannan et al. (2004). Transglucosylation involving p-nitrophenyl β-D-glucopyranoside as donor and β-1-N-acetamido-D-glucopyranose, which is a glycosylasparagine mimic, as acceptor was explored under different reaction conditions using almond β-glucosidase and cloned Pichia etchellsii β-glucosidase II. Both enzymes catalyzed the formation of (1→3)- as well as (1→6)- regioisomeric disaccharides, the former being the major product in cloned β-glucosidase II reaction while the latter predominated in the almond enzyme catalyzed reaction. Use of β-1-N-acetamido-D-mannopyranose and β-1-N-acetamido-2-acetamido-2-deoxy-D-glucopyranose as acceptors in almond β-glucosidase catalyzed reactions, however, did not afford any disaccharide products revealing the high acceptor specificity of this enzyme (Kannan et al., 2004).

The hydrolytic and transglucosidic reactions of the Aspergillus niger Family 3 β-glucosidase were characterized by Seidle and Huber (2005). Advanced analyses indicated have indicated that the substrates became transglucosidic acceptors when present at high concentrations. The study has also showed that gentiobiose forms by an intermolecular reaction of the C6 hydroxyl of Glc rather than an intramolecular reaction and that an equatorial orientation of the C2 hydroxyl, the presence of a C6 primary hydroxyl and β-linkages with oligosaccharide acceptors are important for acceptor reactivity (Seidle and Huber 2005).
2.4.6. Catalytic mechanism of β-glucosidase

All family 1 β-glucosidases share a general mechanism for the hydrolysis of the β-glycosidic linkage between an anomeric carbon and glycosidic oxygen. There are two steriochemically-different hydrolytic mechanisms proposed for cleavage of the β-linkage between the glycone and aglycone parts of a β-glucoside. These mechanisms differ with respect to retention and inversion at the anomeric center of the reduced sugar residue. The β-glucosidases, together with most cellulases and xylanases are known to hydrolyze the substrate while retaining the anomeric configuration of the sugar moiety (Sinnott, 1990; Clarke et al., 1993). The retaining mechanism involves acidic catalysis that protonates the substrate and leads to formation of a transition state oxocarbonium ion. The orientation of the catalytic group is thought to be complementary to anomeric configuration of sugar moiety. The acidic site (i.e. the carboxyl group) interacts with the site where glycosidic oxygen is found. At the end of the cleavage, the anomeric carbon of the monosaccharide reacts with a water molecule to generate β-D-glucopyranose. This mechanism closely resembles the double displacement mechanism proposed for lysozyme (Fig. 5) (Koshland, 1953; Clarke et al., 1993). The inverting mechanism of the anomeric configuration is based on single displacement by a nucleophilic water molecule. The difference between retention and inversion of the anomeric configuration is that only one transition state occurs in the inversion step of the single displacement mechanism. The final product in the inverting mechanism is α-D-glucopyranoside (Sinnott, 1990). Withers and Street (1989) proposed the double displacement mechanism for β-glucosidase catalysis based on NMR studies. It has been elucidated that the substitution of hydroxyl group at C-2 of the sugar by electronegative fluorine destabilized the adjacent positive charge at the transition state (Withers and Street, 1989), resulting in decreased rates of glycosyl-enzyme formation and hydrolysis.
Figure 5: Proposed mechanism for hydrolysis of β-glycosidic bond by the “retaining” β-glycosidases (Wang and Withers, 1995).

This incorporation brought about considerable acceleration of glycosyl-enzyme formation without affecting the rate of glycosyl enzyme hydrolysis (Withers and Street, 1989). In contrast, the β-glycoside-fluorine resulted in retardation of deglycosylation step of the hydrolysis. The glycosyl enzyme intermediate was trapped easily in a transition state, thereby allowing the mechanism of β-glucosidase catalysis to be examined. Withers and Street (1989) also suggested
that a covalent glycosyl enzyme intermediate was formed during the formation of oxocarbonium ion in the transition state.

Formation of the enzyme-substrate complex in β-glucosidase catalyzed reaction is not well understood. Two pathways have been proposed for the transition state of β-glucosidase in the double displacement mechanism: endocyclic and exocyclic pathways. Both the pathways require two amino acid residues in the active site, one serving as a proton donor and the other as a nucleophile. In case of the exocyclic pathway, the carboxylic acid of the amino acid protonates the exocyclic oxygen (the glycosidic oxygen) of the substrate to make it a good leaving group. This gives rise to an unfavorable electronic geometry. The alternative pathway, endocyclic, involves protonation of the endocyclic oxygen in the formation of the enzyme-substrate complex (Clarke et al., 1993). However, Withers and Street (1988) have suggested that these pathways may not be mutually exclusive and that β-glycosidases may act via either the endo or exocyclic pathway depending on the substrate. Once the transition state reached, β-glucosidases hydrolyzed the substrate while the configuration of the transition state was maintained.

In retaining and inverting mechanisms, at least two carboxyl groups are considered to participate in the catalysis of glycosyl hydrolases (Fig. 6). In inverting enzymes, these residues show an average distance of 9.3 Å. However, in retaining enzymes, these two residues are separated by 5.0 Å. In the retaining mechanism, the acidic group (glutamic acid) in the active site donates a proton to glycosidic oxygen and a nucleophilic group facilitates the bond breaking by attacking to C1 of glucose. Of these, the acid-base catalyst glutamic acid is in the motif TFNEP while the nucleophilic glutamic acid is in the motif I/VTENG.

Hydrolysis of the β-glycosidic bond releases the aglycone part and a water molecule provides a proton to a base catalyst (glutamic acid) and the OH group to the covalent bond between the glycone and the enzyme, releasing the glycone.
and regenerating the nucleophilic glutamic acid. The active-site nucleophile has been identified using 2'-4'-dinitrophenyl-2-deoxy-2-fluoro-β-D-glucopyronoside, which is a mechanism-based inhibitor of the enzyme (Withers et al., 1990; Lawson et al., 1996).

![Inverting Mechanism Diagram](image-1)

![Retaining Mechanism Diagram](image-2)

Figure 6: Mechanisms of retaining and inverting glycosidases (Sinnott, 1990).

Three-dimensional structures of several glycosyl–enzyme complexes have been determined through X-ray crystallographic analysis, revealing identities of important amino acid residues involved in the catalysis. In particular involvement of the carbonyl oxygen of the catalytic nucleophile in strong hydrogen bonding to the sugar 2-hydroxyl for the β-retainers or in interactions with the ring oxygen for α-retainers has been revealed by Withers (2001). The glucose ring in the “-1” (cleavage) site in the intermediates formed on several cellulases and a β-glucosidase adopts a normal 4C1 chair conformation. By contrast the xylose ring at this site in a xylanase gets substantially distorted into a
B boat conformation, a significant stereoelectronic implication. Substantial distortion has also been observed in the substrate upon binding to several β-glycosidases, this time to a 1S skew boat conformation. Much less distortion is seen in the substrate bound on an α-transglycosylase. Finally, an efficient catalyst for synthesis, but not hydrolysis, of glycosidic bonds has been generated by mutation of the glutamic acid catalytic nucleophile of a β-glucosidase to an alanine. When used with α-glucosyl fluoride as a glycosyl donor, along with a suitable acceptor, oligosaccharides up to five sugars in length have been made with yields of up to 90% on individual steps (Withers, 2001).

2.5. Selected plants of the study

In this study, the medicinal plants were selected after some short of screening based on β-glucosidase enzyme profile and their possible glycosidic functional-relation. Consequently, the selected medicinal plants for the study are; Withania somnifera (Solanaceae), Andrographis paniculata (Acanthaceae), and Silybum marianum (Asteraceae). Brief reviews on these plants are given below.

2.5.1. Withania somnifera L. (Dunal)

Withania somnifera is a widely used plant in the traditional Ayurvedic medicine system of India, commonly know as “Ashwagandha” (or winter cherry). The plant belongs to the family “Solanaceae”. Withania is an active ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, aphrodisiac, the elderly, and during pregnancy (Chatterjee and Pakrashi, 1995). Many pharmacological studies have been conducted to investigate the properties of ashwagandha in an attempt to authenticate its use as a multi-purpose medicinal agent. In Ayurvedic medicine there is a class of herbs, including Withania somnifera, known as adaptogens or vitalizers. Adaptogens cause
adaptive reactions to disease, are useful in many unrelated illnesses, and appear to produce a state of nonspecific increased resistance (SNIR) to adverse effects of physical, chemical, and biological agents (Singh et al., 1982).

![Chemical structures of Withania somnifera constituents]

Figure 7: Major chemical constituents of *Withania somnifera*.

They are relatively innocuous, have no known specific mechanism of action, normalize pathological effects, and are usually glycosides or alkaloids of a plant. The chemistry of *Withania somnifera* has been extensively studied and over 35 chemical constituents have been identified, extracted, and isolated. The
biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X). Glycosides are the main compounds of interest regarding study on β-glucosidases, seven new withanolide glycosides called withanosides I, II, III, IV, V, VI, and VII from Withania somnifera were illucidated by Matsuda et al. (2001).

The effectiveness of ashwagandha in a variety of rheumatologic conditions may be due in part to its anti-inflammatory properties. Withania somnifera was found to cause considerable reduction in inflammation (Anbalagan and Sadique, 1981). Studies conducted on the mechanism of action for the anti-inflammatory properties on rats showed a decrease in absorption of $^{14}$C-glucose in rat jejunum. Glucose absorption was maintained at the normal level by both Withania somnifera and the cyclooxygenase inhibitor oxyphenbutazone. Both drugs produced anti-inflammatory effects. Similar results were obtained in parallel experiments using $^{14}$C-leucine absorption from the jejunum. These studies suggest cyclooxygenase inhibition may be involved in the mechanism of action of Withania somnifera (Somasundaram et al., 1983). The use of Withania somnifera in treating various forms of cancer, the antitumor and radiosensitizing effects was investigated in urethane-induced lung adenomas in adult male albino mice (Singh et al., 1986). Withania somnifera was also found to act as a radio- and heat sensitizer in mouse S-180 and in Ehrlich ascites carcinoma. Antitumor and radiosensitizing effects of withaferin (a steroidal lactone of Withania somnifera) were also seen in mouse Ehrlich ascites carcinoma in vivo. The studies of Devi et al. (1995) are suggestive of antitumor activity as well as enhancement of the effects of radiation by Withania somnifera.

Glycosides of Withania somnifera (sitoindosides VII and VIII) exhibited significant antistress activity in forced swimming induced immobility in mice; restraint stress induced gastric ulcers in rats, restraint-induced auto-analgesia in
rats, restraint stress effect on thermic response of morphine in rats, and morphine-induced toxicity in aggregated mice (Bhattacharya et al., 1987).

Figure 8: Structures of Withanosides deriveatives from Withania somnifera.
The brain and nervous system are relatively more susceptible to free radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species. The brain also uses nearly 20% of the total oxygen supply. Free radical damage of nervous tissue may contribute to neuronal loss in cerebral ischemia and may be involved in normal aging and neurodegenerative diseases, e.g., epilepsy, schizophrenia, Parkinson’s, Alzheimer’s, and other diseases. Since traditional Ayurvedic use of *Withania somnifera* has included many diseases associated with free radical oxidative damage, it has been considered likely the effects may be due to a certain degree of antioxidant activity. The active principles of *Withania somnifera*, sitoindosides VII-X and withaferin-A (glycowithanolides), have expressed antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase, catalase, and glutathione peroxidase levels in the rat brain frontal cortex and striatum. Decreased activity of these enzymes leads to accumulation of toxic oxidative free radicals and resulting degenerative effects. This implies that *Withania somnifera* does have an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties (Bhattacharya *et al.*, 1997). Total alkaloid extract of *Withania somnifera* roots has been hown its effects on the central nervous system. It exhibited a taming effect and a mild depressant (tranquilizer) effect on the central nervous system in monkeys, cats, dogs, albino rats, and mice. It had no analgesic activity in rats but increased Metrazol toxicity in rats and mice, amphetamine toxicity in mice, and produced hypothermia in mice. Effects of sitoindosides VII-X and withaferin from roots of *Withania* were studied on brain cholinergic, glutamatergic and GABAergic receptors in male Wistar rats (Schliebs *et al.*, 1997). Root extract of *Withania somnifera* was showed immunomodulatory effects in three myelosuppression models in mice: cyclophosphamide, azathioprin, or prednisolone. Significant increases in hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight were observed in WS-treated mice compared to
untreated control mice. Interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) production was also markedly decreased. The major activity of *Withania somnifera* may be the stimulation of stem cell proliferation. *Withania somnifera* reduced CTX-induced toxicity and may prove useful in cancer chemotherapy which needs to confirm the hemopoetic effect with other cytotoxic agents and to determine its usefulness as an adjuvant in cancer chemotherapy (Ziauddin *et al.*, 1996).

2.5.2. *Andrographis paniculata* (Burm. F.) Wall. Ex Nees

*Andrographis paniculata* (AP), the Kalmegh is commonly as "King of Bitters," belongs to the plant family Acanthaceae. It has been used for centuries in Asia to treat gastro-intestinal tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases. It is evident from Indian Pharmacopoeia that it is a prominent member of at least 26 Ayurvedic formulations. Whereas in Traditional Chinese Medicine, *Andrographis* is an important "cold property" herb which is used to treat the heat of body in fevers, and to dispel toxins from the body and to prevent and treat common colds.

A number of diterpenoids and diterpenoid glycosides have been reported containing similar carbon skeleton, mainly andrographolide, neoandrographolide, deoxyandrographolide and several others are 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, andrographiside, deoxyandrographiside, homoandrographolide, andrographan, andrographon, andrographosterin and stigmasterol (Siripong *et al.*, 1992). The leaves of *Andrographis* contain the highest amount of andrographolide, the most medicinally active phytochemical in the plant, while the seeds contain the lowest (Sharma *et al.*, 1992). The structure of andrographolide contains (i) an α-alkylidene c-butyrolactone moiety, (ii) two olefin bonds Δ8(17) and Δ12(13), and (iii) three hydroxyls at C-3, C-19, and C-14. Of the three hydroxyl groups, the one
at C-14 is allylic in nature, and the others at C-3 and C-19 are secondary and primary, respectively. Matsuda et al. (1994) revealed six new diterpenoids of ent-labdane type, 14-epi-andrographolide, isoandrographolide, 14-deoxy-12-methoxyandrographolide, 12-epi-14-deoxy-12-methoxyandrographolide, 14-deoxy-12-hydroxyandrographolide and 14-deoxy-11-hydroxyandrographolide as well as two new diterpene glucoside, 14-deoxy-11,12-didehydroandrographi-side and 6′-acetylneoandrographolide, and four new diterpene dimers, bisandrographolide A, B, C and D, were isolated along with six known compounds. Phytochemical investigation of the roots and aerial parts of Andrographis paniculata yielded a new flavone, 5-hydroxy-7,20,60-trimethoxyflavone and an unusual 23-carbon terpenoid, 14-deoxy-15-isopropylidenene-11,12-didehydroandrographolide together with five known flavonoids and four known diterpenoids and one deoxyandrographolide-19β-D-glucoside.

Studies on mice showed that Andrographis paniculata is a potent stimulator of the immune system in two ways: (i) Antigen-specific response: antibodies are made to counteract invading microbes, and (ii) Nonspecific immune response: macrophage cells scavenge and destroy invaders. AP activates both responses - making it effective against a variety of infectious and oncogenic (cancer-causing) agents (Puri et al., 1993). The initial interest with AP has its hepatoprotective (liver protecting), as well as its anti-cancer properties; similarly, it has an effective treatment in immune boosting.

Andrographis have great promising effect on viability of the HIV virus; andrographolide prevented transmission of the virus to other cells and stopped the progress of the disease by modifying cellular signal transduction. Andrographolide probably does this by inhibiting enzymes that facilitate the transfer of phosphates. Andrographolide may inhibit HIV-induced cell cycle dysregulation, leading to a rise in CD4+ lymphocyte levels in HIV-1 infected individuals (Holt et al., 1998).
Figure 9: Major chemical constituents of *Andrographis paniculata*.
The effect in common cold, fever, and inflammation was observed for all major andrographolides: deoxyandrographolide, andrographolide, neoandrographolide, and dehydroandrographolide. Dehydroandrographolide had the most pronounced effect, followed by neoandrographolide and andrographolide. The anti-inflammatory effect seemed to work by a mechanism that involved the adrenal glands. Further study confirmed that the anti-inflammatory action of dehydroandrographolide was due to its effect on increasing the synthesis and release of adrenocorticotropic hormone (ACTH) of the pituitary gland of the brain. ACTH signals the adrenal gland to make cortisol, a natural anti-inflammatory (Yin and Guo, 1993).

Extracts of AP containing the four major active components were evaluated for antimalarial activity against *Plasmodium berghei*, one of the parasites that transmit malaria. The extract was found to produce considerably inhibition of multiplication of the parasites (Misra *et al.*, 1992). The effects of AP indicated the protective action due to reactivation of superoxide dismutase (SOD). AP extracts are also effective in killing filaria (microscopic worms) that obstructs lymph channels in the body, leading to gross swelling termed elephantiasis. Since no toxic effects were apparent, researchers believed that the AP plant extract would be safe for humans and no plant has previously been shown to have antifilarial action. Screening of aqueous extracts of *Andrographis*, andrographolide and arabinogalactan proteins showed significant antibacterial and antifungal activities in comparison to some known antibiotics. The investigations revealed the biological value of the cumulative effects of AP and AD resulting in enhanced antimicrobial activity (Singh *et al.*, 2003).

### 2.5.3 *Silybum marianum* (L.) Gaertn.

*Silybum marianum*, commonly known as “milk thistle” belongs to the family Asteraceae (or Compositae), is a herbaceous annual or biennial plant with a dense prickly flower head with purplish tubular flowers. Milk thistle is an
edible plant native to southern Europe, southern Russia, Asia Minor, and northern Africa, and has been used for food in the countries surrounding the Mediterranean for a long time as well as a tonic herb for the liver. Virtually all parts of the plant have been used as food with no known toxicity. Its seeds and roots have been used for an assortment of medical purposes for thousands of years.

*Silybum* contained a wide range of molecules such as; flavonoids lignans (silymarin comprised of silybin, silydianin, and silychristin), essential oils, flavonoid (quercetin), tannins, and fixed Oil. The mixture of three biochemicals; silychristine, silydianin, and silybin in general, is called "silymarin." The biological mechanism of action is not well known but several theories exist: silymarin may control cell membrane permeability which means that silymarin may control what substances actually enter the interior of a cell; silymarin may inhibit chemical pathways leading to inflammatory biochemicals; silymarin may have free radical scavenging properties which means that it may absorb harmful reactive atoms that could damage other molecules; silymarin may increase protein production by liver cells; silymarin may stabilize mast cells (cells containing inflammatory granules); and, silymarin in higher doses increases the bile-flow (Fraschini *et al.*, 2002).

Some flavonoid glycosides are prepared synthetically or by biotransformations, usually for pharmaceutical purposes. Silybin is a flavonolignan used extensively as a potent hepatoprotectant and an antidote in mushroom poisoning. The only drawback of this compound is low water solubility; therefore its glycosylation was attempted by biological and chemical methods. Silymarin prevents uptake of the poison into the cells of the liver and thus, prevent the lethal liver damage associated with this type of mushroom poisoning. Biotransformation led to the formation of silybin 7-β-glucoside and chemical glycosylations gave silybin glycosides at C-23 (β-glucoside, β-galactoside, β-maltoside and β-lactoside) (Kren *et al.*, 1998).
Figure 10: Structure of major biomoleclues from *Silybum marianum.*
Solubility of the silybin glycosides was improved considerably compared to the aglycone. Biological tests showed that the silybin glycosides have a considerably higher radical scavenging activity. Silybin monoglycosides have also much better hepatoprotective activity than the aglycon in the tests measuring the release of lactate dehydrogenase from hepatocytes intoxicated with t-butylhydroperoxide. Similar effect was observed also in the tests with membrane lipoperoxidation of the mitochondrial membranes where silybin glycosides, mainly β-glucoside and β-galactoside, were found to be better antilipoperoxidants. Cytotoxicity of higher concentrations of silybin towards hepatocytes can be lowered also by its glycosylation - in this case especially β-maltoside and β-lactoside displayed substantially lower toxicity at high concentrations. Similarly, silybin β-galactoside was also found to have better hepatoprotective activities in vivo presumably due to β-galactosyl residue causing higher affinity towards hepatocytes (Kren et al., 1998).

Silymarin is known to protect the liver by altering and strengthening the structure of outer cell membranes of hepatocytes (liver cells), preventing toxins from entering the liver cells, and by stimulating the regenerative ability of the liver and the formation of new hepatocytes through the activation of an enzyme nucleolar polymerase A, which leads to the increase in ribosomal protein synthesis and cell division (Flora et al., 1998). Silymarin, as an anti-oxidant, may also reduce damages to liver cells caused by chronic use of certain prescription drugs. In some studies, Silybum is concerned to hypoglycemia, the patients treated with silymarin did not have any increase in the number of mild or severe hypoglycemic episodes, suggesting that silymarin stabilized as well as lowered glucose levels. In addition, SGOT and SGPT values declined significantly in the patients taking silymarin, confirming that liver function improved. There was also a decrease in blood levels of malondialdehyde, a marker of free radical damage, approaching that of healthy subjects (Flora et al., 1998; Gazak et al., 2004). Application of silybin/silymarin as a chemoprotective and anti cancer
agent could be considered in the first view for its antiradical potential and consequently for its cytoprotective activity. Owing to its chemopreventive effect silybin/silymarin inhibits carcinogenic action of many chemicals and significantly decreased the incidency of urinary bladder neoplasms and preneoplastic lesion in the initiation and post-initiation phase of the induction by N-butyl-N-(4-hydroxybutyl) nitrosamine. This compound also significantly limited azoxymethane-induced colon carcinogenesis in rats. Silymarin inhibited skin carcinogenesis induced by benzoyl peroxide or 12-O-tetradecanoylphorbol-13-acetate. Silybin or silymarin may be useful in treatment and prevention of some neurodegenerative and neurotoxic processes, partly due to its antioxidative activity. An extract from *Silybum marianum* seeds was tested on the differentiation and survival of cultured neural cells (rat PC-12 pheochromocytoma cell line). The extract enhanced the differentiation of PC-12 cells and prevented apoptosis following nerve growth factor (NGF) withdrawal. Moreover, the extract protected primary hippocampal neurons against oxidative stress-induced apoptosis (Kren and Walterova, 2005).

Silybin also interacts with other drug transporters, e.g., with multidrug resistance-associated protein 1 (MRP1). Influence of silymarin and other flavonoids was tested in human pancreatic adenocarcinoma cell line (Panc-1) on the transport of daunomycin and vinblastin. It was found that silymarin significantly increases accumulation of both drugs in the cells indicating the inhibition of MRP1. It seems that GSH regeneration is involved in this process because in the other study with flavonoids stimulation of GSH co-transport, ATPase and drug resistance-conferring properties of MPR1 were found to be modulated (Leslie *et al.*, 2001). Soluble derivatives of the hepatoprotective flavonolignan silybin, namely silybin galactoside, glucoside, lactoside and maltoside were investigated for their radical scavenging and antilipoperoxidation properties. Cyclic voltammetry results showed that glycosides are weaker electron donors than silybin, although it was of interest
that they were found to be more potent scavengers of the 1,1-diphenyl-2-picrylhydrazyl and the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)-derived radicals. The glycosides were more efficient than silybin in preventing tert-butyldihydroperoxide-induced lipoperoxidation of rat liver mitochondrial membranes. Furthermore, glycosides were significantly more cytoprotective than silybin in tert-butyldihydroperoxide-damaged rat erythrocytes and primary hepatocyte cultures. Glycosylation of silybin substantially reduced its toxic effects in primary cultured hepatocytes observed during prolonged incubation. These results suggest that silybin glycosides are suitable soluble derivatives of silybin for experimental studies and may have therapeutic potential (Kosina et al., 2002).

2.6. Fucosyltransferase

An off-shoot of this study on Silybum marianum proteomic analyses has resulted in the identification of a putative fucosyltransferase. Since as such there is little investigation on fucosyltransferases from plants, the relevant literature on them has been presented here holistically on plant and non-plant sources like Arabidopsis thaliana, Oryza sativa, Populus (Populus tremula x Populus alba), and Homo sapiens (Perrin et al., 1999; Sasaki et al., 2002; Costa et al., 2007; Kelly et al., 1995)

Fucosyltransferase catalyzes transfer of L-fucose from a GDP-fucose (Guanosine diphosphate-fucose) donor to an acceptor leading to biosynthesis of fucosides. The acceptor substrate can be another sugar such as the transfer of a fucose to a core GlcNAc (N-acetylglucosamine) sugar as in the case of N-linked glycosylation, or a protein, as in the case of O-linked glycosylation produced by O-fucosyltransferase. Fucosyltransferase activity may be a risk factor for urinary tract infection. GDP-fucose protein O-fucosyltransferase 1 (PoFUT1) is an enzyme responsible for adding fucose sugars in O linkage to serine or threonine residues between the second and third conserved cysteines in EGF-like repeats on the
Notch protein. The protein is an inverting glycosyltransferase, which means that the enzyme uses GDP-β-L-fucose as a donor substrate and transfers the fucose in O linkage to the protein producing fucose-α-O-serine/threonine. Almost all glycosyltransferases reside in the Golgi apparatus. However, PoFUT1 as well as the related enzyme PoFUT2 have recently been shown to reside in the endoplasmic reticulum (Costache et al., 1997).

Plant cell walls play a crucial role in development, signal transduction, and disease resistance. They are made of cellulose and matrix polysaccharides such as hemicelluloses and pectins. Xyloglucan, the principal hemicellulose of dicotyledonous plants, has a terminal fucosyl residue that may affect the extensibility of the cell wall and thus influence plant growth and morphology. A key component in the association between cellulose and xyloglucan is the presence of the L-fucose-containing trisaccharide side-chain. Computer modeling of xyloglucan structure predicts that fucose containing xyloglucans adopt spatial conformations more favorable for cellulose binding than nonfucosylated xyloglucan (Levy et al., 1991). Fucosylated xyloglucans bind cellulose in vitro at a 2-fold higher rate than do nonfucosylated xyloglucans (Levy et al., 1997); however, xyloglucan fucosylation is not absolutely required for the formation of cellulose–xyloglucan networks (Whitney et al., 1995). Fucose-containing xyloglucan is also thought to play a role in the regulation of plant growth. Several studies have shown that xyloglucan-derived oligosaccharides, called oligosaccharins, act as inhibitors of auxin-stimulated elongation of pea epicotyls. The biological activity of these oligosaccharins depends on the presence of terminal L-fucose (Cote and Hahn, 1994).

There are various fucosyltransferases in mammals, the vast majority of which are located in the Golgi apparatus however O-fucosyltransferases have recently been shown to be localized to ER also. Compared to animals, very few fucosyltransferases are known and far little characterized from plant world.