Chapter II - Introduction

2.1. Historic retrospective of Diabetes

Diabetes mellitus clinical features have been known for centuries. Egyptians already mention it as early as 1500 BC, in the Ebers papyrus, where they describe a disease characterized by “passing too much urine”. Although there have been other descriptions in China, Japan and India, associating polyuria with the sweetness of urine, it was not until II century BC that the term “diabetes” emerged. It meant “siphon” and it was Arateus of Capadocia, a Greek physician, who characterized it as “the melting down of flesh and limbs into urine”. Many centuries passed until in 1675, Thomas Willis added the word “mellitus” (honey, sweet) after rediscovering the sweetness of urine and blood of patients. In 1776, Dobson confirmed the presence of excess sugar in urine and blood as the cause of their sweetness (Ahmed, 2002).

Experimental medicine in modern times allowed a more detailed insight into diabetes mellitus so, in 1857, Claude Bernard discovers the role of the liver in glycogenesis and the concept that diabetes is due to an excess of glucose production.

Pancreatic islets were only discovered later by Brockman, but only got their current name when published by Langerhans in 1869 (Krall et al., 1994). The role of pancreas in the pathogenesis of diabetes was revealed by Von Mering and Minkowski in 1889, when, by removing the pancreas in dogs, they verified the establishment of diabetes. At the end of the XIX century, Opie noticed islet beta-cell injury in patients who died from the disease (Cahil, 1985).

An important milestone in diabetes history was the isolation of insulin (Banting et al., 1922). It was in 1921 that Banting and colleagues achieved isolation of insulin from pancreas finding a way to avoid the death of thousands, for which they have received the Nobel Prize in 1923.

In 1936, Himsworth discovers that diabetes falls into two types based on “insulin insensitivity” which later on would lead to the classification of type 1 and 2 diabetes (Himsworth, 1936).
Oral hypoglycemic drugs also began being developed and, by 1955, the first sulfonylurea, carbutamide, was successfully used in clinical trials. Second and third generation insulin secretagogues were successively developed (Leibovitz, 1999). The use of these hypoglycemic agents was later enriched by the utilization of other drugs such as alpha-glucosidases, biguanides and tiazolidenediones (King, 2000; Cusi and De Fronzo, 1998; Leibovitz, 1998), which many are still in use nowadays.

2.2 Diabetes pathophysiology, prevalence and classification

According to the World Health Organization (WHO), Diabetes Mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). The chronic hyperglycemia of diabetes is associated with specific micro and macrovascular chronic complications, resulting in damage to or failure of various organs, notably the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), heart and blood vessels (macrovascular diseases) and an overall diminished quality of life (WHO, 2006a).

Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 (Wild et al., 2004) (Figure 2.1). The mortality rate in patients with diabetes may be up to 11 times higher than persons without the disease (Modi, 2007). The American Diabetes Association (ADA) estimated the national costs of diabetes in the USA for 2002 to be $US 132 billion, increasing to $US 192 billion in 2020 (American Diabetes Association, 2003).
Normal glucose homeostasis depends on three factors:

i) the ability of some tissues to take up glucose in response to insulin (insulin sensitivity);

ii) the ability of cells to take up glucose in the absence of insulin, sometimes referred to as glucose sensitivity, and;

iii) the ability of the pancreatic beta-cell to control this process by both rapid and sustained insulin secretion (first- and second-phase insulin secretion) (Khan et al., 1996).
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The vast majority of cases of diabetes fall into two broad etiopathogenetic categories: type 1 and type 2. Type 1 diabetes onset is usually acute, developing over a period of a few days to weeks. Its prevalence accounts for 5-10% of the diabetic people and over 95 percent of those persons develop the disease before the age of 25 with an equal incidence in both sexes and an increased prevalence in the white population (Mayfield, 1998). It results from beta-cell death following viral/autoimmune attack. Therefore, type 1 diabetes is characterized by failure of pancreatic beta-cells to produce and secrete insulin and it is treated by insulin replacement therapy (Salway, 2004).

As for type 2, it is the most common form of diabetes mellitus, including 90–95% of the diabetic subjects. It is highly associated with a family history of diabetes, older age, obesity and lack of exercise. Pathophysiologically, patients with type 2 diabetes, exhibit insulin resistance and usually have relative (rather than absolute) insulin deficiency (American Diabetes Association, 2010). Onset of insulin resistance is often accompanied by obesity, namely visceral obesity. Resistance of dysfunctional fat cells to the antilipolytic effects of insulin leads to chronic elevations in plasma of free fatty acid levels. This, in turn, induces insulin resistance in the liver and skeletal muscle, resulting in reduced glucose uptake and increased gluconeogenesis (Staels and Fruchart, 2005; Salway, 2004). So it is postulated that the insulin produced is relatively ineffective probably due to altered beta-cells, skeletal muscle, adipose tissue or liver function (Salway, 2004).

Other specific types of diabetes mellitus of various known etiologies are grouped together. This group includes:

i) persons with genetic defects of beta-cell function or with defects of insulin action;

ii) persons with diseases of the exocrine pancreas, such as pancreatitis or cystic fibrosis;

iii) persons with dysfunction associated with other endocrinopathies (e.g., acromegaly) and;

iv) persons with pancreatic dysfunction caused by drugs, chemicals or infections (Report of the Expert Committee on the Diagnosis and
Classification of Diabetes Mellitus, 1997).

Due to the expanding prevalence rates of type 2 diabetes, both in developed and developing nations, the prevention of type 2 diabetes assumes global importance. The fact that there is a long delay between development of the earliest metabolic defects and full expression of the disease, type 2 diabetes lends itself to potential preventive action. Therefore, lifestyle modification and/or pharmacologic intervention that can ameliorate insulin sensitivity (reduce insulin resistance) or improve/preserve beta-cell function would expect to be of extreme importance on the future development of type 2 diabetes (Crandall et al., 2008).

2.2.1. Type 2 diabetes disease progression

In most individuals there is a low progression from normal to impaired glucose tolerance (IGT) to frank diabetes. This depends on interactions between genetic and environmental factors that act on both initiation and progression of the disease (Khan, 1996). In the early stages of type 2 diabetes mellitus, insulin resistance can be compensated by an increase in insulin secretion leading to normal glucose tolerance. As we can see in Figure 2.2, with increasing insulin resistance, the fasting plasma glucose will rise, accompanied by an increase in fasting plasma insulin levels, until a fasting plasma glucose level is reached when the beta-cell is unable to maintain its elevated rate of insulin secretion at which point the fasting plasma insulin declines sharply. Hepatic glucose production will begin to rise. When fasting plasma glucose reaches high levels, the plasma insulin response to a glucose challenge is markedly blunted (Scobie, 2007).

The American Diabetes Association has encouraged the use of the term impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) to recognize an intermediate group of individuals whose glucose levels do not meet criteria for diabetes and yet are higher than those considered normal. These people were defined as having impaired fasting glucose (IFG) when IFG levels vary from 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L), or impaired glucose tolerance (IGT) when 2-h values in the oral glucose tolerance test (OGTT) are between 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0
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Clincially, impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG) represent intermediate conditions in the transition between normality and frank diabetes: an essentially asymptomatic but still potentially pathological stage characterized by mild hyperglycemia. People with IGT or IFG are at high risk of progressing to type 2 diabetes, although this is not inevitable (WHO, 2006b).

Treatment of type 2 diabetes is complicated by several factors inherent to the disease process, typically, insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin-mediated glucose uptake and utilization (Tiwari and Rao, 2002).
2.2.2. Glucose homeostasis and insulin signal transduction – sites of impairment in type 2 diabetes

After the breakdown of carbohydrates by digestive enzymes, the released glucose becomes the primary stimulus for beta-cells of the pancreatic islets. Prolonged exposure of pancreatic islets to elevated glucose concentrations has been shown both in vitro and in vivo to impair glucose-stimulated insulin release. Glucose stimulation of pancreatic islet beta cells initiates a cascade of events resulting in insulin secretion (Figure 2.3) (Tiwari and Rao, 2002). Initially glucose enters beta-cells through the high capacity glucose transporter type 2 (GLUT-2) and is phosphorylated by glucokinase. The generation of ATP from glycolysis increases the intracellular ATP/ADP ratio. ATP binds to ATP-dependent potassium channels on the beta-cell membranes closing these channels and depolarizing the cell membranes. The depolarization activates voltage sensitive calcium channels causing a calcium influx triggering insulin secretion (Cazarolli, 2008).

![Figure 2.3. Glucose stimulated insulin secretion. The 'classical' pathway involves glucose transport by means of glucose transporter 2, sensing (by glucokinase) and subsequent metabolism (by glycolysis) to generate ATP, which leads to the closure of ATP-sensitive K⁺ channels. This depolarizes the plasma membrane, opening voltage-dependent Ca²⁺ channels, allowing Ca²⁺ to enter the cell and trigger insulin secretion.](image-url)
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channels, which leads to an influx of extracellular $\text{Ca}^{2+}$ and thus results in insulin exocytosis. The sulphonylurea receptor (SUR) is the binding site for sulphonylureas that act as insulin secretagogues (Lowe, 1998).

As it can be observed in Figure 2.4, once insulin is released by beta-cells the molecular signalling is mediated by a complex mechanism of action. In insulin-sensitive tissues and in the presence of this hormone, the activated insulin receptor phosphorilates the insulin receptor substrate proteins (IRS proteins), which are linked to the activation of signalling pathways: the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B(PKB) pathway, which is responsible for most of metabolic actions of insulin; and the Ras-mitogen-activated protein kinase (MAPK) pathway, which regulates expression of some genes and cooperates with the PI3K pathway to control cell growth and differentiation. Alternatively, through specific phospholipase C (PLC) activity, a second messenger: inositol phosphate glycan (IPG), can be produced which activates the protein phosphatases which in turn regulate glucose and lipid metabolism (Cazarolli, 2008).

Figure 2.4. Insulin signal transduction (Adapted from Cazaroli, 2008).
Glucose enters eukaryotic cells via two types of membrane-associated carrier proteins, the Sodium/glucose cotransporters (S-GLUTs) and the facilitative glucose transporters (GLUTs) (Table 2.1). There are several facilitative glucose transporters (GLUTs), each one with different tissue distributions, kinetic properties and sugar specificity. The GLUT-1 transporter is widely expressed in various tissues and is responsible for basal glucose uptake. The GLUT-2 isoform is primarily expressed in beta-cells and in the liver, and has a relatively low affinity for glucose which in combination with hexokinase serves as part of glucose sensor. GLUT-3 has the highest affinity and is expressed during fetal development and in adult neurons. As for GLUT-4, it is predominantly present in fat and muscle and is responsible for insulin-stimulated glucose uptake. This process involves insulin receptor tyrosine kinase and the primary consequence is GLUT-4’s translocation from intracellular storage sites to the plasma membrane. In the basal state, GLUT-4 is localized in intracellular vesicles, while in the presence of insulin, GLUT-4 is localized in the plasma membrane of fat, skeletal and cardiac muscle (Cazarolli, 2008). S-GLUTs, on the other hand, constitute a large family of membrane proteins involved in the transport of glucose, amino acids, vitamins, osmolytes, and some ions across the brush border membrane of the small intestine epithelium and the renal proximal tubules. These kind of transporters concentrate glucose inside the cells, using the energy provided by contranport of sodium ions down their electrochemical gradients. S-GLUT-1 and S-GLUT-2 proteins are believed to be the most important ones (Sabino-Silva et al, 2010; Hediger and Rhoads, 1994).
Table 2.1. Main glucose transporters, tissue distribution and type of transport (International Chair on Cardiometabolic Risk: www.cardiometabolic-risk.org).

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Organ</th>
<th>Type of transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT-1</td>
<td>Erythrocytes</td>
<td>Passive</td>
</tr>
<tr>
<td></td>
<td>Blood-brain barrier</td>
<td></td>
</tr>
<tr>
<td>GLUT-3</td>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td>GLUT-5</td>
<td>Intestine</td>
<td></td>
</tr>
<tr>
<td>GLUT-2</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>GLUT-4* / GLUT-1</td>
<td>Adipose tissue</td>
<td></td>
</tr>
<tr>
<td>GLUT-4* / GLUT-1</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>GLUT-2</td>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>S-GLUT-1</td>
<td>Intestine</td>
<td>Active</td>
</tr>
<tr>
<td>S-GLUT-2</td>
<td>Kidney epithelial cells</td>
<td></td>
</tr>
</tbody>
</table>

* Insulin sensitive

This complex system of insulin-stimulated whole-body glucose utilization is impaired in people that develop insulin resistance and type 2 diabetes. Type 2 diabetes is polygenic and may involve polymorphisms in multiple genes encoding the proteins involved in insulin signalling, insulin secretion and intermediary metabolism. Defects, genetic or acquired, emerge at many levels, with decreases in receptor concentration and kinase activity, the concentration and phosphorylation of IRS-1 and -2, PI3K activity, glucose transporter translocation, and the activity of intracellular enzymes. Activation of the MAPK pathway by insulin is not reduced in type 2 diabetes, perhaps allowing for some of the detrimental effects of chronic hyperinsulinaemia on cellular growth in the vasculature (Saltiel and Khan, 2001).

2.2.3 Type 2 diabetes current treatments

Type 2 diabetes patients often require oral agents or insulin for glucose control. At present, at least five different major forms of therapy are available for type 2 diabetes: lifestyle modifications of diet and exercise, insulin replacement therapy, insulin secretagogues, insulin sensitizers and alpha-glucosidase inhibitors. To achieve good glucose control and prevent complications of diabetes, patients usually are on multiple
drug combinations. There is, therefore, great diversity in terms of the response and tolerance to the current medications (Modi, 2007).

**Insulin secretagogues - Sulfonylureas**

Sulfonylureas have been available for many years and are classified as either first-generation agents such as acetohexamide, chloropropamide and tolbutamide (1) or as second-generation agents like glimepiride, glipizide and glyburide (2). The second-generation agents are more potent and have better pharmacokinetic and safety profiles (Modi, 2007).

Sulfonylureas lower fasting plasma glucose primarily by increasing the release of insulin from functioning pancreatic beta-cells. They display a glucose-dependent effect. With long-term use, there is a progressive decrease in the effectiveness of sulphonylureas, which appears to be related to exhaustion of beta-cell function. Sulfonylureas increase plasma levels of insulin and cause hypoglycemia. Hypoglycemia and weight gain are two most frequent side effects of these drugs (Modi, 2007; Rendell, 2004).

**Insulin secretagogues - Non-sulfonylureas**

The metiglinide analogs, including nateglinide (3) and repaglinide (4), are non-sulfonylurea insulin secretagogues that also bind to potassium ATP channels, albeit at a different site than traditional sulfonylureas. These agents stimulate the release of insulin from pancreatic beta-cells if glucose is present. Metiglinide analogs have much shorter half-lives than do sulfonylureas. Nateglinide mimics physiologic insulin secretion dynamics seen in healthy individuals by increasing early phase insulin secretion into the portal vein and in that way increases hepatic glucose uptake as well as hepatic glucose suppression. Clinical trials have demonstrated that nateglinide can reduce post-prandial hyperglycemia and therefore improve glycemic control. Both nateglinide and repaglinide reduce fasting plasma glucose and help decrease some side effects such as hypoglycemia, hyperinsulinemia, weight gain, and possible beta-cell exhaustion characteristic of the sulfonylureas (Philips and Dunning, 2003; Bloomgarden, 1997).
**Insulin sensitizers - Biguanides**

In contrast to the sulfonylureas, biguanide metformin (5) does not stimulate insulin secretion. The precise mode of action of metformin remains somewhat controversial, but its predominant effect is to reduce hepatic glucose production in the presence of insulin (Inzucchi, 2002). Besides that, it sensitizes the liver to circulating insulin levels and increases glucose uptake in the skeletal muscle and peripheral tissues (Reedy and King, 2011). It is useful for patients who are obese as it does not promote weight gain as seen with sulfonylureas. It is as capable as sulfonylureas to lower glycosylated haemoglobin (HbA1c). Metformin also exerts a positive effect upon lipid metabolism and it reduces blood trygliceride, LDL cholesterol and fatty acid levels (Wulffele et al., 2004). Adverse effects of metformin therapy include gastrointestinal distress, such as abdominal pain, nausea, and diarrhea, in up to 50% of patients (Bailey and Turner, 1996).

**Insulin sensitizers - Thiazolidinediones**

Thiazolidinediones (TZDs) are another class of hypoglycemic agents used in diabetes treatment, which target the regulation of adipocyte function in order to improve insulin sensitivity. They are pharmacological ligands for a nuclear receptor known as peroxisome proliferator-activated receptor gamma (PPAR gamma), which require insulin in order to work. Activation of these receptors regulates the transcription of insulin-responsive genes that regulate carbohydrate and lipid metabolism (Mudaliar and Henry, 2001). TZDs may also improve beta-cell function by reducing free fatty acids. TZDs are effective in reducing HbA1c. Examples of TZD’s include troglitazone (6) that has a lipid lowering effect and increases HDL and pioglitazone (7) that also decreases triglycerides (Nagai et al., 2001). The major side effects of TZDs are associated with edema and weight gain. (Modi, 2007)

**Alpha-glucosidase inhibitors**
The alpha-glucosidase inhibitors, that include acarbose (8) and miglitol (9), slow down the rate of carbohydrate absorption in the small intestine, which results primarily in a reduction in postprandial plasma glucose levels. Their mechanism of action is unique, and this is the sole drug class not targeted at a specific pathophysiological defect of type 2 diabetes mellitus. They act as competitive, reversible inhibitors of alpha-glucosidase and alpha-amilase enzymes (Inzucchi, 2002). These agents do not affect insulin levels, so they do not cause hypoglycemia when used alone. Gastrointestinal adverse effects are common, affecting up to 30% of patients (Modi, 2007).

A summary of the chemical structures of some of the drugs, reviewed above, is represented in Figure 2.5 and their corresponding targets and effects can be observed in Figure 2.6.

<table>
<thead>
<tr>
<th>Type 2 Diabetes Current Treatments</th>
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<tbody>
<tr>
<td><strong>Insulin secretagogues - Sulfonylureas</strong></td>
<td></td>
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<tr>
<td>Tolbutamide (1)</td>
<td>Glyburide (2)</td>
</tr>
<tr>
<td><img src="image1" alt="Tolbutamide" /></td>
<td><img src="image2" alt="Glyburide" /></td>
</tr>
<tr>
<td><strong>Insulin secretagogues - Non-sulfonylureas</strong></td>
<td></td>
</tr>
<tr>
<td>Nateglinide (3)</td>
<td>Repaglinide (4)</td>
</tr>
<tr>
<td><img src="image3" alt="Nateglinide" /></td>
<td><img src="image4" alt="Repaglinide" /></td>
</tr>
</tbody>
</table>
### Insulin sensitizers - Biguanides

<table>
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<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure" /></td>
<td>Metformin (5)</td>
</tr>
</tbody>
</table>

### Insulin sensitizers - Thiazolidinediones

<table>
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<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image2.png" alt="Structure" /></td>
<td>Troglitazone (6)</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure" /></td>
<td>Pioglitazone (7)</td>
</tr>
</tbody>
</table>

### Alpha-glucosidase inhibitors

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image4.png" alt="Structure" /></td>
<td>Acarbose (8)</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure" /></td>
<td>Miglitol (9)</td>
</tr>
</tbody>
</table>

**Figure 2.5.** Examples of different drugs used for type 2 diabetes treatment.
Recent alternatives

**Dual peroxisome proliferator-activated receptor agonists**, such as Dual PPAR alpha/gamma agonists that are currently in development, are set to combine the properties of thiazolidinediones with fibrates (Staels and Fruchart, 2005). Both PPAR alpha and gamma agonists exert renal protective effects in type 2 diabetic animals which supports the idea that their may have additive or synergistic benefits on metabolic control and vascular complications in type 2 diabetes (Cha et al., 2007). This combination is therefore likely to broaden the therapeutic value of these agents, holding considerable promise for improving the management of type 2 diabetes and providing an effective therapeutic option for treating the multifactorial components of cardiovascular disease and the
metabolic syndrome (Staels and Fruchart, 2005).

**Peptide analogs - Incretin mimetics**

*Exenatide* and *liraglutide* are incretin mimetics that have been shown in human studies to be an effective treatment to improve glycemic control in patients with type 2 diabetes. They display biological properties similar to human gut polypeptide called glucagon-like peptide-1 (GLP-1). These substances decrease a person’s appetite and slow down gastric emptying leading to weight loss. Moreover, they enhance glucose-dependent insulin secretion by the pancreatic beta-cells, as well as increase the ability of the body to dispose of extra blood glucose after meals. Long-term, they may even promote new production of beta-cells in the pancreas. Reported adverse effects of exenatide and liraglutide include nausea, vomiting, and transient headache, as well as increased risk of hypoglycemia when used with sulfonylureas. (Modi, 2007; De Fronzo et al., 2005; Joy et al., 2005).

**Dipeptidyl peptidase-4 inhibitors**, are a type of hypoglycemic agent that helps the body respond to glucose from a meal by increasing insulin levels and adjusting other hormones (Mody, 2007). The mechanism of action is thought to result from increased incretin levels which inhibits glucagons release and therefore increases insulin secretion. They can also contribute to beta-cell mass preservation (McIntosh et al., 2005).

**Amylin analogs**

*Pramlintide acetate*: is a synthetic version of human amylin, a hormone produced along with insulin by the beta-cells in the pancreas. Pramlintide acts by delaying gastric emptying and inhibiting the release of glucagon (Jones, 2007; Modi, 2007).

The reason that, even after so much advancement in understanding the disease process and availability of a wide range of therapeutic agents, the disease is still progressing may be due to the fact that individual agents act only on part of the
pathogenic process and only to a partial extent (Tiwari and Rao, 2002). In spite of the
great diversity and specificity that current treatments provide, they do not satisfactorily
control blood glucose levels, which is the primary purpose, nor do they prevent diabetes
complications (Modi, 2007).

2.3. The value of plants used in traditional medicine for drug discovery

There is a wide historical record of the use of plants in medicine, based upon the
tradition of different cultures. Nowadays it is estimated that over 80% of the world’s
population uses plant materials as their source of primary health care (Farnsworth et al.,
1985). The science involved in the study of the pharmacological aspects and agents
involved in a culture's medical treatment is designated as ethnopharmacology.

This science emerged, as an independent field of research, fairly recently in the late
70’s (Rivier and Bruhn, 1979) when it was described as a highly diversified approach to
drug discovery involving the observation, description, and experimental investigation of
indigenous drugs and their biologic activities. It consists of a multidisciplinary field based
on botany, chemistry, biochemistry, pharmacology, and many other disciplines
(anthropology, archaeology, history, and linguistics) that contributes to the discovery of
natural products with biologic activity.

The main goals of using plants as sources of therapeutic agents have been:

i) to isolate bioactive compounds for direct use as drugs,

ii) to obtain bioactive compounds of novel or known structures as lead compounds
for semisynthesis in order to produce patentable entities of higher activity and/or
lower toxicity,

iii) to use them as pharmacologic tools and,

iv) to use the whole plant or part of it as a herbal remedy (Fabricant and Farnsworth,
2001).

Considering the number of organisms, the number of interactions is almost infinite,
and thus an enormous wide variety of secondary metabolites has been developed in the
course of evolution. Nature is thus a very important source of new leads for drug
development (Schmidt et al, 2007; Verpoorte and Alfermann, 2000). Ethnopharmacology
is therefore an important tool because around three quarters of biologically active plant-derived compounds presently in use worldwide have been discovered through follow-up research to verify the authenticity of data from folk and ethnomedicinal uses (Lewis and Elvin-Lewis, 1995; Farnsworth et al., 1985). Medicinal plant derived products present an advantage in this area; based on their long-term use by humans (often hundreds or thousands of years), one might expect any bioactive compound obtained from such plants to have low human toxicity (Fabricant and Farnsworth, 2001).

Drug discovery using higher plants can be accomplished through different approaches:

i) random selection followed by chemical screening;

ii) random selection followed by one or more biological assays;

iii) biological activity reports and ethnomedical use of plants.

The latter approach includes plants used in traditional medical systems: herbalism, folklore, and shamanism; and the use of databases. The main aim is to isolate new bioactive phytocompounds. When an active extract has been detected, the first step is to identify the bioactive phytocompounds, which may mean either a full identification of a bioactive phytocompound after purification or partial identification to the level of a family of known compounds (Mendonça-Filho, 2006).

2.4. Plant secondary metabolism

Secondary metabolites were previously regarded as waste products but are now recognized to provide interesting biological activities, namely, by providing resistance against diseases (Verpoorte, 1998).

Secondary metabolism arises by modification of existing primary metabolic reactions (Vining, 1992). Primary metabolism leads to the formation of a pool of organic compounds of low molecular weight and simple structures such as carboxylic and amino acids, which are vital for living organisms. They form the synthetic materials for specific, genetically controlled, and enzymatically catalyzed reactions that lead to the complex compounds that characterize the secondary metabolism of plants and animals (Ikan, 2007).
Although natural product’s chemodiversity is immensely vast, in practice and following the biogenetic classification (Ikan, 2007), 6 main classes are defined (Hanson, 2003): Polyketides and fatty acid-derived substances; Terpenoids and steroids; Phenylpropanoids; Alkaloids; Specialized amino acids and peptides; Specialized carbohydrates.

The building blocks for secondary metabolites are derived from primary metabolism as indicated in Figure 2.7. Metabolites from the fundamental processes of photosynthesis, glycolysis and the Krebs cycle are tapped off from energy-generating processes to provide biosynthetic intermediates. The most important building blocks employed in the biosynthesis of secondary metabolites are derived from the intermediates acetyl coenzyme A, shikimic acid, mevalonic acid, and 1-deoxyxylulose 5-phosphate. These are utilized respectively in the acetate, shikimate, mevalonate and deoxyxylulose phosphate pathways (Dewick, 2002).
2.4.1. **Polyphenols**

Polyphenols constitute secondary metabolic products that arise biosynthetically from phenylalanine via two main pathways in plants: the shikimic pathway and the acetate pathway (Heller, 1985). Flavonoids are the most abundant sub-class of polyphenols and currently more than 6000 different structures have been identified (Aherne and O’Brien, 2002). They share a common nucleus (Figure 2.8) consisting of a benzene ring A, condensed with a six member ring C to which a phenyl benzene ring is connected at position 2 (or 3 in the case of isoflavones).
Introducción

Flavonoids can be further divided into 6 sub-classes on account of the variations in the heterocyclic C-ring. Ring C may be a heterocyclic pyran, which yields flavanols (catechins) and anthocyanidins, or pyrone, which yields flavonols, flavones, isoflavones and flavanones (Figure 2.9) (Kuhnau, 1976). The patterns of substitution on the flavan nucleus include hydrogenation, hydroxylation, malonylation, methylation, and sulfation (Harborne, 1986). While most flavonoids occur naturally attached to sugar moieties (glycosides) such as glucose, rhamnose, galactose, arabinose, and thus tend to be water-soluble, a small fraction do not carry any sugar group and are termed aglycones (Harborne, 1986).

Flavonoids occur richly in seeds, olive oil, fruits, tea, herbs, and wine where they provide much of the flavour and colour that accompany these products (Middleton et al. 2000). Polyphenols, in general, are not only used by plants to establish symbiotic interactions with other organisms, they are also used for other types of communication with their environment and for defense (eg. most herbivores avoid consuming levels of phenolics that are in excess of their normal diet, especially when tannins are involved). For example, they protect plants from UV radiation and give plants their coloring patterns important for pollination purposes (Harborne and Williams, 2000; Dixon and Paiva, 1995; Waterman and Mole, 1994).

![Flavonoid basic structure](image-url)
2.4.2 Flavonoid biosynthetic pathway

The flavonoid pathway is part of the larger phenylpropanoid pathway, which produces a range of other secondary metabolites, such as lignins, lignans, and stilbenes. The key flavonoid precursors are phenylalanine, obtained via the shikimate pathway, and malonyl-CoA, derived from citrate produced by the Krebs cycle (Figure 2.10).
Introduction

Shikimate → R=H; L-Phenylalanine
               R=OH; L-Tyrosine

↓

PAL (Phenylalanine ammonia lyase)

Lignins/Lignans
Coumarins
Phenylpropanoids

R=H; Cinnamic acid
R=OH; 4-Coumaric acid

Malonyl-CoA → 3x

CHS

Stilbenes

Chalcone

Aurone

Isoflavone

Flavanone

Flavone

Flavanonol

Flavonol

Catechins
Anthocyanins
Figure 2.10. The phenylpropanoid pathway by which plants synthesize a wide range of flavonoids. Chalcone synthase (CHS) is the first step in the branch of the pathway that produces the flavonoids including, flavanones, aurones, isoflavones, flavones, flavanonols, flavonols and anthocyanins (Adapted from Crozier, 2000). PAL: Phenylalanine ammonia lyase.

Most flavonoids are derived from phenylalanine via cinnamic and p-coumaric acids by the addition of three malonate units and subsequent cyclization. Phenylalanine ammonia lyase (PAL) catalyzes the first step of the biosynthetic process. The next major enzyme in the series, p-coumarate: CoA ligase catalyzes the addition of three malonyl-CoA units. The intermediate is then cyclized via a Claisen condensation to produce a chalcone intermediate catalyzed by chalcone synthase (CHS) (Figure 2.11.).

Chalcones are key intermediates in the formation of several major groups of flavonoids. In some plants, they are converted into aurones (Chapter 3, Figure 3.10). In other instances, chalcones undergo reduction of the exocyclic double bond to produce dihydrochalcones (Seigler, 1998).

![Chalcone formation mechanism](image)

Figure 2.11. Mechanism proposed for chalcone formation through enzymes CoA ligase and Chalcone synthase.

Chalcones and flavanones exist in equilibrium in vitro systems (Chapter 3, Figure 3.11). Although this isomerization can occur chemically, acid conditions favouring flavanone and basic conditions the chalcone, but in nature the reaction is enzyme catalyzed and stereospecific, resulting in formation of a single flavanone enantiomer the S-enantiomer (Dewick, 2002).
Flavanones can then originate many variants on this basic skeleton (flavones, flavonols, anthocyanins). Desaturation of flavanones can therefore yield flavones, whereas introduction of oxygen at the 3-position gives dihydroflavonols (flavanonols), which in turn, are the precursors of flavonols, anthocyanins and condensed tannin precursors. Modifications to the hydroxylation patterns in the two aromatic rings may occur, generally at the flavanone or dihydroflavonol stage, and methylation, glycosylation and dimethylallylation are also possible, increasing the range of compounds enormously (Seigler, 1998).

2.5. Polyphenols role in human body

Polyphenols cannot be produced by the human body and have to be taken in through the daily diet. The human diet consists of a large part of plant-derived products, like vegetables, fruits, and tea. These food products contain relatively high levels of polyphenols. Therefore, the total average intake of polyphenols in a healthy human diet has been estimated to be around 1 g per day (Scalbert and Williamson, 2000).

The evidence reported that phenolics, namely flavonoids, play a vital biological role, including the function of scavenging reactive oxygen species (Gupta et al., 2010). Chemically, there are three features that grant flavonoids their remarkable antioxidant properties (Rice-Evans et al., 1997):

i) the hydrogen donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids, which enable the flavonoids to undergo a redox reaction that helps them to scavenge free radicals more easily;

ii) a stable delocalization system, consisting of aromatic and heterocyclic rings as well as multiple unsaturated bonds, which helps to delocalize the resulting free radicals and;

iii) the presence of certain structural groups such as the catechol structure in the B-ring and also the 2,3-double bond conjugated with the 4-oxo-group and the 3-OH group, which are capable of forming transition metal-chelating complexes that can regulate the production of reactive oxygen species such as hydroxyl radicals and oxygen radicals (Figure 2.12 and 2.13) (Peng et al., 2003; Pietta, 2000).
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**Figure 2.12.** Scavenging of ROS (R\(^\bullet\)) by flavonoids (Fl-OH). Displayed is the catechol group of the B-ring. The aroxyl radical, acquiring a stable quinine structure (Adapted from Pietta, 2000).

**Figure 2.13.** Example of metal ion/flavonoid complexation. The proposed binding sites for trace metals to flavonoids are: catechol moiety of B-ring, 3-OH, C4=O and C4=O, 5-OH groups (Adapted from Pietta, 2000).

### 2.5.1. Polyphenols of plant origin used for type 2 diabetes prevention or treatment

Despite the great interest in the development of new drugs to revert the burden of complications associated with diabetes mellitus its incidence is still considered to be high (Pickup and Williams, 1997). It is also a major cause of disability and hospitalization and it results in the raised interest of the scientific community to evaluate either raw or isolated natural products, as hypoglycemic agents. There has been a large number of crude plant extracts and purified substances from plants tested (*in vitro* and *in vivo* preclinical trials) for treatment of diabetes although only a few of them have been tested in humans (Johnson et al., 2006; Jung, et al., 2006; Vuksan and Sievenpiper, 2005; Lieu et al., 2004).
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In epidemiologic studies some flavonoids have been related to aging disease protection. That can be justified due to the antioxidant action of this type of compounds (Thabrew et al., 1998). As antioxidants, polyphenols can protect cells against oxidative stress, limiting therefore the risk of several degenerative diseases associated with oxidative stress, such as cardiovascular disease, neurodegenerative diseases and diabetes (Giugliano et al., 1996).

2.5.2 Polyphenols main targets in glucose metabolism

Oxidative stress as a consequence of hyperglycemia, in association with decreased cell antioxidant defense system and increase of radical oxygen species (ROS) play an important role in diabetes physiopathology (McCune et al., 2006).

Oxidative stress is also potentially responsible for diabetes complications pathology. One of the most serious complications is cardiovascular disease. Prevalence of cardiovascular disease in diabetic patients is from 3 to 4 times higher than in non-diabetic subjects (Jialal et al., 2002). Nephropathy is a microvascular complication typical of diabetes and is present in one third of the insulin-dependent diabetic patients, and has its origin indirectly related to hyperglycemia, that through protein kinase activation increases oxidative damage of renal cells (Kedziora-Kornatowska et al., 2000). Another complication that derives from diabetes is neuropathy in which hyperglycemia is central for its development and progression. One of the mechanisms through which hyperglycemia causes neuronal degradation is, precisely through an increase of oxidative damage (Baydas et al., 2003).

In a diabetic state, the elevated blood glucose concentration promotes, at several stages of glucose metabolism, an increase of the oxidative stress. Flavonoids have been shown to inhibit ROS production by inhibiting several ROS producing enzymes and by chelating trace metals. They act by donating a hydrogen atom/electron to the superoxide anion and also to hydroxyl, alkoxyl and peroxyl radicals thereby protecting lipoproteins, proteins as well as DNA molecules against oxidative damage (Dembinska-Kiec et al., 2008).

Besides their clear in vitro antioxidant activity, polyphenols also possess various other actions on diabetes therapy, namely the capacity to lower blood glucose levels,
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acting as hypoglycemic or anti-hyperglycemic agents. As we can observe from Figure 2.14, polyphenols can act at several levels during glucose absorption and utilization.

It has been described that polyphenols can act since the beginning of the digestive process. When complex carbohydrates are degraded by enzyme alpha-amylase present in the saliva, and also by the membrane-bound alpha-glucosidase present in the small intestine, polyphenols can interfere with glucose absorption, inhibiting those enzymes. Intestinal glucose and fructose absorption can also be affected by polyphenols that act by inhibiting glucose transporters such as the sodium-dependent glucose transporter (S-GLUT-1) or GLUT-5, respectively. After glucose is absorbed into the blood stream, beta-pancreatic cells, sensitive to blood glucose levels, proceed to secrete insulin. At this point polyphenols may interfere inducing insulin secretion and/or affecting beta-cell proliferation or apoptosis. We can verify insulin receptor activation, which will unroll a sequence of reactions at the peripheral tissues, leading them to absorb glucose through
glucose-transporter GLUT-4. Polyphenols can affect glucose uptake into the peripheral tissues, through activation or inhibition of GLUT-4. Another stage where polyphenols have demonstrated activity is at the polyol pathway where they have been shown to inhibit aldose reductase enzyme, preventing sorbitol accumulation in the cell.

2.5.2.1. Polyphenols as glucose transport or absorption inhibitors

Several studies report the potential of antidiabetic medicinal plants on inhibition of carbohydrate hydrolysing enzymes, alpha-amylase and alpha-glucosidase and manipulation of glucose transporters. It is envisaged therefore that there are several approaches to retard glucose uptake in the small intestine:

- by inhibiting digestive enzymes,
- by inhibiting active transport of glucose across intestinal brush border membrane, and,
- by delaying the gastric emptying rate of gastrointestinal content.

The alpha-glucosidase inhibitors are currently the most commonly used oral agents for ameliorating post prandial hyperglycemia (PPHG) because of the lack of hypoglycemic threat, and more importantly because of the prospect of blood glucose control without hyperinsulinemia and body weight gain (Tiwari and Rao, 2002).

**Quercetin** (10), a flavonol, at 0.5 mM has shown activity at glucose absorption level by presenting *in vitro* porcine pancreatic alpha-amylase inhibitory activity with an IC$_{50}$ value lower than 500 µM (Tadera et al., 2006). At glucose transporter level this compound (10-20 µM) is a good GLUT-2 inhibitor (in oocytes) with an IC$_{50}$ of 22.8 µM. *In vivo*, when administered (60 mg/Kg) to transgenic diabetic rats, glucose absorption inhibition was observed (Song et al., 2002). **Isoquercitrin** (11), (0.4-0.8 mM) a quercetin glycoside, inhibited glucose transporter S-GLUT-1 present in the intestine (Cermak et al., 2004). *In vivo*, using normal rats, the post prandial peak was delayed by 30 minutes when administering orally at 100 mg/Kg, showing therefore glucose absorption inhibition (Paulo, et al., 2008).

**Myricetin** (12), another flavonol, at 0.5 mM have shown potent porcine pancreatic alpha-amylase inhibitory activity with an IC$_{50}$ of 380 µM (Tadera et al., 2006).
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Epicatechin gallate (13) and epigallocatechin gallate (14) abundant in green tea and tested at 1 mM, using brush-border membrane vesicles obtained from the rabbit small intestine, inhibited S-GLUT-1 in a competitive manner, although epicatechin gallate itself was not transported via S-GLUT-1 leading to believe that it interacts with S-GLUT-1 as an antagonistic-like molecule (Kobayashi et al., 2000). Other catechins, epigallocatechin (15) and gallocatechin (16) have demonstrated inhibition of rat intestinal alpha-glucosidase and porcine pancreatic alpha-amylase with IC$_{50}$ of 739 and 401 µM, respectively (Bhandari et al., 2008).

Luteolin (17), a flavone, at 0.5 mM have shown potent porcine pancreatic alpha-amylase inhibitory activity with an IC$_{50}$ of 360 µM (Tadera et al., 2006). In another study not only luteolin (17) but also luteolin-7-O-glucoside (18) (0.5 mg/mL) have been reported to have inhibitory activities on alpha-glucosidase and alpha-amylase (Kim et al., 2000), therefore possibly suppressing post-prandial hyperglycemia in patients with type 2 diabetes mellitus.

Also flavones, scutellarein (19), scutellarein-7-O-glucoside (20), scutellarein-7-O-(6-O-feruloyl)-glucoside (21), luteolin-7-O-glucoside (18), and luteolin-7-O-(6-O-feruloyl)-glucoside (22) have shown rat intestinal alpha-glucosidase inhibitory activity at 500 µM (Kawabata et al., 2003).

In an *in vivo* study, isoflavone glycoside sissotrin (23), when administered orally to normal rats at 100 mg/Kg, impaired glucose tolerance presenting an increased post prandial glycemic peak, showing therefore, no glucose absorption inhibition (Paulo, et al., 2008). On the contrary and in the same study, phlorizin (24), a dihydrochalcone, when administered (100 mg/Kg; orally) to normal rats has shown a time-dependent antihyperglycemic activity by delaying the post-oral glucose load glycemic peak at 30 minutes (Paulo et al., 2008). Phlorizin has been known for long to be a selective S-GLUT-1 inhibitor and therefore usefull as antidiabetic agent in the treatment of non-insulin-dependent diabetes mellitus (Khan et al., 1991). For instance, when administered (400 mg/Kg/day; 4 weeks treatment; subcutaneously) to partially pancreatectomized diabetic rats, completely normalized insulin sensitivity although discontinuation of phlorizin treatment in phlorizin-treated diabetic rats resulted in the reemergence of insulin
resistance. Additionally, phlorizin administration was associated with reversal of first- and second-phase insulin secretory defects in this mode (Rossetti et al., 1987).

**Naringenin (25)**, a flavanone, exhibited moderate inhibitory action on glucose uptake in rabbit intestinal brush border membrane vesicles, and showed strong inhibitory action in rat everted intestinal sleeves. The IC$_{50}$ values were 205.9 and 2.4 µM respectively. *In vivo*, naringenin (500 µM) also showed reduction in the glucose uptake by more than 60% in both the intestinal and renal brush border membrane vesicles of diabetic rats reaching levels similar to that of the normal rats (Li et al., 2006).

Polyphenolic compounds, **caffeic acid (26)** (1mM), **ferulic acid (27)** (1mM), **chlorogenic acid (28)** (1mM) and **tannic acid (29)** (1mg/mL) pre-treatment of brush border membrane vesicles from rat intestine, promoted an inhibitory effect on S-GLUT-1 mediated glucose transport (Welsh et al., 1989).

### 2.5.2.2. Polyphenols with insulin secretagogue action

A proper pancreatic function implies a well regulated insulin secretion. Several studies describe the effects of polyphenols in insulin secretion.

**Quercetin (10)**, has been shown to normalize glucose tolerance of STZ-diabetic animals at doses of 10-15 mg/Kg/day (intraperitoneally) during 10 days. Quercetin treatment also increased hepatic glucokinase activity, probably by enhancing insulin release from pancreatic islets (Vessal et al., 2003). It also exerts stimulatory effect on insulin secretion at different concentration range 10-100 µM and in presence of 20 mM glucose (Hii et al., 1985; Pérez et al., 1998).

The quercetin glycoside, **rutin (30)**, when orally administered (25-100 mg/Kg) to STZ-induced diabetic rats for 45 days induced an increase in insulin secretion (Kamalakkannan and Prince, 2006) with a decrease in fasting blood glucose levels. At these same concentrations rutin also exhibited antioxidant capacity by exhibiting decreased values of glycosilated hemoglobin as well as tiobarbituric acid reactive species and lipidic hydroperoxides (Kamalakkannan and Prince, 2006).
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Flavanol, **epicatechin** (31) (80µM) has been shown to be an insulin secretion stimulatory compound and this flavonoid may, at least in part, exert its effects on insulin release via interference with calcium beta-cell influx (Hii et al., 1985). Flavanols such as **catechin** (32) (20-200 µM) and **afzelechin** (33) (10 nM-10 µM) when tested in pancreatic MIN6 cell line, revealed a dose-dependent increase in insulin release. It is possible that catechin’s antioxidant properties, by preventing glucose toxicity and ameliorating islet cell dysfunction, contribute to its insulin secretagogue activity. As for afzelechin, that induced a larger insulin release at much lower concentrations there might be other mechanisms involved (Chemler et al., 2007).

Flavone, **apigenin glycoside** (34) (Apigenin-6-C-(2’’-O-rhamnosyl)-fucoside) was found to have an acute effect on blood glucose lowering when administered orally (50 mg/Kg) to diabetic rats and stimulated glucose-induced insulin secretion after oral treatment in hyperglycemic rats (Cazarolli, et al., 2009). **Isovitexin** (35) and **swertisin** (36), two C-glycosylflavones administered orally at the dose of 15 mg/Kg, showed clear acute antihyperglycemic effect in hyperglycemic rats as well as stimulated significantly insulin secretion *in vivo* (Folador et al., 2010).

Also, the isoflavone **genistein** (37), when applied in vitro (10-100 µM) to normal mice pancreatic islets, presented increased insulin secretion (Jonas et al., 1995).

Some anthocyanins like **cyanidin-3-O-glucoside** (38) and **delphinidin-3-O-glucoside** (39) and **pelargonidin** (40) (50 µg/mL) presented insulin secretagogue activity when tested in INS-1 pancreatic cell line (Jayaprakasan et al., 2005). Similar compounds such as **leucopelargonidin glycoside** (dimethoxy ether of leucopelargonidin-3-O-rhamnoside) (41) also showed significant hypoglycemic, hypolipidemic and serum insulin-raising effects in moderately diabetic rats with close similarities to the effects of glibenclamide (Cherian et al., 1993). Additionally when orally administered at 100 mg/Kg to normal and to moderately alloxan-induced diabetic dogs, it showed significant hypoglycemic and serum insulin raising action during a period of two hours (Augusti et al., 1994).

**Pterostilbene** (42), has been shown to increase insulin secretion when administered (40 mg/Kg/daily for 45 days) to Streptozotocin (STZ)-Nicotinamide-
induced diabetic rats (Amarnath et al., 2006).

4-Hydroxybenzoic acid (43) showed an acute hypoglycemic action when administered orally (5 mg/Kg) to normal Wistar rats. The mechanism involved an increase in serum insulin levels and glycogen content in the liver (Peungvicha, et al., 1998).

2.5.2.3. Polyphenols with insulin mimetic action or with insulin sensitizing action

It is well established that insulin regulates glucose homeostasis by modulating the activities of protein kinases in its target tissues: muscle, liver and fat. Impairment in insulin signalling leads to insulin resistance which in combination with deficient insulin secretion from the pancreas results in hyperglycemia, typical in diabetic subjects (Coghalan and Smith, 2005). Therefore, molecules that selectively modulate protein kinase activities in insulin-resistant tissues may act either as insulin-sensitizing or insulin-mimetic drugs and could be useful for treating type 2 diabetes.

Quercetin (10) and kaempferol (44), flavonols, tested at 20-50 µM presented a significant improvement in insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes as well as significant inhibitory effects on nitric oxide (NO) production in response to lipopolysaccharide treatment in macrophage cells in which the PPAR gamma (peroxisome proliferators-activated receptor gamma) was overexpressed. These flavonols can, therefore, potentially act at multiple targets to ameliorate hyperglycemia, including by acting as partial agonists of PPAR gamma (Fang et al., 2008). PPAR gamma is a nuclear transcription factor that has been implicated in several disease pathologies, namely diabetes. PPAR gamma phosphorylation state modulates insulin sensitivity. In another study, kaempferol, tested at 10 µM, when in contact with macrophages significantly induced the PPAR gamma expression by 7.66-fold. Besides, it also increased PPAR gamma activity in a dose dependent manner with an EC$_{50}$ of approximately 10 µM (Liang et al., 2001).

Also, kaempferitrin (45) (kaempferol-3,7-O-dirhamnoside) showed hypoglycemic activity when administered at 100 mg/Kg (52 mM) to alloxan-induced diabetic rats. The same study determined that the hypoglycemic activity derived from stimulation of
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glucose uptake by 43 and 46 %, by the soleus muscle cells when applying 52 and 104 mM of kaempferitrin (Jorge et al., 2004). Another kaempferol glycoside, kaempferol-3-neohesperidoside (46) (1-100 nM) has shown insulinomimetic effects by stimulating glucose uptake in rat soleus muscle (Zanatta et al., 2008).

The flavonol myricetin (12) has been shown to stimulate lipogenesis in adipocytes (EC$_{50}$ = 65 µM) increasing the effect of insulin and therefore, acting as an insulinomimetic (Ong and Khoo, 1996). In vivo, when administered (0.3-1.0 mg/Kg; intravenously) to STZ-induced diabetic rats also demonstrated an increase in glucose utilization in the absence of insulin (Liu et al., 2005).

Catechin (32), was given orally at 5, 10 and 20 mg/Kg for 6 weeks to STZ-induced diabetic rats and an antihyperglycemic effect was observed. At 20 mg/Kg, catechin markedly increased tissue glycogen, and 14C-glucose oxidation without any change in plasma insulin and C-peptide. Catechin also restored the altered glucokinase, glucose-6 phosphatase, glycogen synthase and glycogen phosphorylase levels to near normal. GLUT4 mRNA and protein expression in skeletal muscle were enhanced after catechin treatment (Daisy et al., 2010).

Epigallocatechin gallate (14), a green tea flavanol, has a glucose lowering effect in animals. It is reported to decrease hepatic glucose production and increase tyrosine phosphorylation of the insulin receptor substrate-1 (IRS-1) and mimicking insulin by increasing the PI3 and MAP Kinase activity (Waltner-Law et al., 2002). In another study, the same compound enhanced glucose uptake in rat skeletal muscle cells by a PI3 kinase-mediated mechanism (Jung et al., 2008) which is in accordance with what has recently been reported; that it promotes GLUT4 translocation in skeletal muscle cells (Ueda et al., 2008).

The flavones, chrysin (47) and apigenin (48), when in contact with macrophages at 10 µM significantly induced the PPAR gamma expression by 8.13- and 5.60-fold, respectively. PPAR gamma activity increase was also observed in a dose dependent manner with a EC$_{50}$ of approximately 5 and 10 µM (Liang et al., 2001). Apigenin glycoside (Apigenin-6-C-(2''-O-rhamnosyl)-fucoside) (34) presented a significant stimulatory effect on glucose uptake in rat soleus muscle at tested concentrations, 50 and
100 μM. Other two flavone glycosides, rhoifolin (49) and cosmosin (50), were tested in differentiated 3T3-L1 adipocytes and showed dose-dependent response in concentration range (0.001–5 μM and 1–20 μM), enhanced adiponectin secretion (an important endocrine secreted cytokine which affect insulin sensitivity through modulation of insulin signaling pathway), tyrosine phosphorylation of insulin receptor-b and GLUT4 translocation (Rao et al., 2009).

Isoflavone genistein (37) presents an immediate glucose uptake inhibition in adipocytes with a GLUT-4 IC_{50} of 20 μM (Bazuine et al., 2005). However, genistein, and soy isoflavonoids when given as isoflavone-rich diet for 11 weeks to obese zucker rats significantly improved lipid and glucose metabolism. Additionally, genistein (9.3 μM) along with daidzein (51) (9.8 μM) increased PPAR alpha and gamma-directed gene expression, therefore these isoflavones are probably exeriting their antidiabetic activities as PPAR agonists (Mezei et al., 2003). Puerarin (52) (daidzein-C-glucoside), another isoflavone, when administered to obese rats (75 mg/Kg) inhibits glucose uptake in the intestine. Daidzin (53) (daidzein-O-glucoside), on the other hand, at same conditions stimulates glucose uptake in the intestine (Meezan et al., 2005). In another experiment, an acute intravenous administration of puerarin (15 mg/Kg) to STZ-induced diabetic rats, significantly decreased blood glucose levels. Besides, repeated puerarin administration (15 mg/Kg; 3 times daily) for 3 days to STZ-diabetic rats, increased the GLUT4 protein level at the rats soleus muscle. Therefore puerarin can increase the glucose utilization to lower plasma glucose in diabetic rats lacking insulin (Hsu et al., 2003).

The isoflavonoids dehydroeugenol (58), glyasperin D (59), dehydroglyasperin C (60), dehydroglyasperin D (61), glycyrin (62) and glycycoumarin (63) exhibited significant PPAR-gamma ligand-binding activity (Prabkhar and Doble, 2008) so they probably can improve insulin sensitivity. Additionally, glycyrin (62) administered in the food at 100 mg/Kg/day for 4 days, significantly decreased the blood glucose levels of genetically diabetic mice, possibly because of its considerable PPAR gamma-ligand binding activity (Kuroda et al., 2003).

4-hydroxyderricin (54) and xantoangelol (55), two chalcones showed two types of insulin-like activities: (5-20 μM) potentiated 3T3-L1 pre-adipocytes differentiation
into adipocytes and enhanced glucose uptake but without PPAR gamma activation. *In vivo*, using genetically diabetic KK-Ay mice, these chalcones (0.15% of each chalcone in powdered diet; 4 weeks) were able to prevent blood glucose levels elevation, promoting a 50% and 33% reduction respectively (Enoki et al., 2007).

Sakuranetin (56), a flavanone, when incubated (10-100 µM) with 3T3-L1 adipocytes enhanced adipogenesis and at 100 µM increased glucose uptake, increasing therefore adipocyte insulin sensitivity (Saito et al., 2008).

Treatment with the stilbene resveratrol (57) for 15 weeks increased both insulin-stimulated whole-body and steady-state glucose uptake of both soleus muscle and liver in high cholesterol-fructose-fed rats (Deng et al., 2008).

Mangiferin (64) (30-90 mg/Kg) and mangiferin–7-O-glucoside (65) (90 mg/Kg), are two xantones that were tested for antidiabetic activity in KK-Ay mice and have shown to lower the blood glucose levels after 3 weeks of oral administration improving, simultaneously, hyperinsulinemia. The antidiabetic activity observed was shown to be due to decreased insulin resistance (Miura et al., 2001a; Miura et al., 2001b).

### 2.5.2.4. Polyphenols effect on beta-cell mass preservation

Complex diseases, such as diabetes, lead to loss of functional beta cell mass. Beta cell apoptosis can be a common feature of type 1 and type 2 diabetes, although the mechanism for its activation may be different. Some flavonoids have shown antiapoptotic effects in pancreatic cells. On the other hand, pancreatic integrity can also be achieved by regeneration of the endocrine pancreas and although there aren’t many studies performed in pancreatic beta cells, flavonoids have shown to modulate cell proliferation in other cell types (Pinent et al., 2008).

Quercetin (10), administered intraperitoneally at 10-15mg/Kg/day for 10 days to STZ-induced diabetic rats, increased the number of pancreatic islet cells (Vessal et al., 2003). In a more recent study, using a similar STZ-induced diabetic model, the quercetin treated group (25 mg/Kg/day intraperitoneally; 30 days) showed a significant decrease in elevated blood glucose, malonaldahyde and nitric oxide levels. Furthermore, there was a significant increase in antioxidant enzyme activities, as well as pancreatic insulin
Quercetin treatment protected and preserved pancreatic beta-cell architecture and integrity (Adewole et al., 2007). In another study which also involved quercetin, a complete protection against IL-1beta- and IFN-gamma-mediated cytotoxicity in pancreatic RIN cells was achieved. This compound have also inhibited inducible NO synthase (iNOS) gene expression and prevented IL-1beta- and IFN-gamma-mediated inhibition of insulin secretion. According to the authors the molecular mechanism implicated may involve the inhibition of nuclear factor kappa B (NF-kB) activation (Kim et al., 2007b).

Also, kaempferol (44), another flavonol, when tested at 10 µM in 2-deoxy-D-ribose-damaged HIT-T15 pancreatic cells suppressed oxidative damage reducing intracellular ROS, apoptosis, and lipid peroxidation (Lee et al., 2010).

Earlier reports had mentioned the protective effect of a polyphenol on beta pancreatic cells, one of which dates from 1981 when Chakravarty and colleagues tested flavanol epicatechin (31) in alloxan-induced diabetic rats and realized that it possessed beta-cell preventive as well as restorative properties against alloxan-induced damage. (Chakravarty et al., 1981) More recently, epigallocatechin gallate (EGCG) (14) tested at up to 200 µg/mL during 24h (co-treatment) prevented cytokine-induced cell death both in insulinoma cell line (Han, 2003) and in isolated mice islet cells (Song et al., 2003) in a dose-dependent manner. A possible explanation could be by down-regulation of inducible nitric oxide synthase (iNOS) expression through inhibition of nuclear factor kappa B (NFkB) activation (Han, 2003). In another study, EGCG (10-100 µM) reduced hypoxia-reperfusion-induced apoptosis as well as decreased the markers of oxidative damage (Hara et al., 2006).

Flavones such as apigenin (48), and luteolin (17) have also presented cytoprotective effects by exhorting a complete protection against IL-1beta- and IFN-gamma-mediated cytotoxicity in RIN cells. These compounds have also inhibited inducible NO synthase (iNOS) gene expression and prevented IL-1beta- and IFN-gamma-mediated inhibition of insulin secretion. According to the authors the molecular mechanism implicated may involve the inhibition of nuclear factor kappa B (NF-kB) activation (Kim et al., 2007c).
Dietary intake of isoflavone genistein (37) significantly improved hyperglycemia, glucose tolerance, and blood insulin levels in streptozotocin-induced diabetic mice, concomitant with improved islet beta-cell proliferation, survival, and mass (Fu and Liu, 2007). Genistein (5 µM/24h) induced both INS1 pancreatic cells and human islet beta-cell proliferation and the mechanism by which it modulated pancreatic beta-cell function was via activation of the cAMP/PKA-dependent ERK1/2 signaling pathway (Fu et al., 2010). In another study genistein has also been shown to modulate beta-cell apoptosis. In pancreatic islets and in beta-cell line, low doses (25 µM) reduced NaF-induced apoptosis whereas high (100 µM) caused apoptosis (Elliot et al., 2002). Again, genistein (5–40 µM/3h pre-incubation) has presented cytoprotective effects, by preventing cytokine-induced NO production, iNOS expression, ERK-1/2 and JAK/STAT activation as well as avoiding glucose-stimulated insulin secretion impairment. Collectively, these results suggest that genistein might be used to preserve functional beta-cell mass (Kim et al., 2007a). Another isoflavone, puerarin (52) (50-100 µM), significantly suppressed H₂O₂-induced apoptosis in rat islet cells as well as increased antioxidant enzymes activities (Xiong et al., 2006).

Sulforuretin (66), an aurone, inhibited cytokine-or STZ-induced cell damage in rat insulinoma RINm5F and in isolated rat islets (40-100 µM; 3h preincubation) by reduction of iNOS expression, nitric oxide production and normalization of insulin secretion in response to glucose. In vivo, sulforuretin by intraperitoneal administration (40 µg/Kg/daily for 3 days, prior to STZ injection) completely prevented diabetogenic effects of STZ in mice (Song et al., 2010).

Silymarin (67), a flavonolignan, has shown a direct cytoprotective effect by preventing cytokine-induced apoptosis when tested at 100 µM in RINm5F insulinoma cell line. Additionally, cytokine-induced iNOS expression and NO production were inhibited and both proteins: Jun-N-terminal kinase (JNK) and signal transducer and activator of transcription (STAT) were activated in human islets (Matsuda et al., 2005).

2.5.2.5. Polyphenols as aldose reductase inhibitors
Aldose reductase, the key enzyme of the polyol pathway, has been demonstrated to play an important role in the etiopathology of diabetic complications such as neuropathy, cataract, nephropathy and retinopathy. Aldose reductase catalyses the reduction of glucose into sorbitol which does not readily diffuse across the cell membrane and its intracellular accumulation is responsible for cataract in diabetic patients. The inhibitors of aldose reductase have been proved to improve the diabetic complications in experimental animals and clinical trials (Tiwari and Rao, 2002).

Flavonols, quercitrin (10), guaijaverin (68) and desmanthin-1 (69) showed the most potent rat aldose reductase inhibitory activity: an IC\textsubscript{50} of 0.15, 0.18 and 0.082 \(\mu\)M respectively, the last of them was equivalent to commercial synthetic aldose reductase inhibitor, epalrestat (IC\textsubscript{50}=0.072 \(\mu\)M) (Matsuda et al., 2002b).

The ethyl acetate fraction of \textit{Salicornia herbacea}, when administered orally at 25 mg/Kg to STZ-induced diabetic rats (4, 7 and 24 h hour after diabetes induction) caused a decrease in serum glucose levels and also a decrease in sorbitol accumulation in the lenses, red blood cells and sciatic nerves. The main compound responsible for this effect was identified as isorhamnetin-3-O-glucoside (70), a flavonol which presented rat lens aldose reductase inhibitory activity, with an IC\textsubscript{50} of 1.4 \(\mu\)M (Lee et al., 2005).

Isoaffinetin (71) (5,7,3’,4’,5’-pentahydroxyflavone-6-C-glucoside) showed potent inhibition of porcine lens aldose reductase with an IC\textsubscript{50} of 4.6 \(\mu\)M (Haraguchi et al., 2003).

The chalcone butein (72) was identified as the most promising antioxidant and aldose reductase inhibitor for prevention and treatment of diabetic complications (Lim et al., 2001). Particularly when administering butein to STZ-diabetic rats (75mg/Kg/twice a day) for two weeks, aldose reductase inhibitory activity was observed, with nearly 70% decrease in sorbitol accumulation in sciatic nerve (Lim et al., 2001).

Acylated flavanone glucoside myrciatrin IV (73) has been reported to possess potent aldose reductase inhibition activity with an IC\textsubscript{50} of 0.79 \(\mu\)M (Matsuda et al., 2002a).
Also, the phenylpropanoids lithospermic \(74\) and rosmarinic acid \(75\), tested \textit{in vitro} at 100 \(\mu\)M have shown 95% aldose reductase inhibitory activity (Koukoulitsa et al., 2006).

\textbf{2.5.2.6. Polyphenols as antioxidants \textit{(in vivo)}}

Postprandial hyperglycemic episodes in diabetic patients are closely associated with increased oxidative stress, and are a very important factor in the onset and progress of vascular complications, both in type 1 and 2 diabetes mellitus. Moreover, recent studies show that majority of the plasma antioxidants are depleted in type 2 diabetes patients (Valabhji et al., 2001). It, therefore, became clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications (Johansen et al., 2005). Studies on the use of antioxidants as potential adjunct therapies in type 2 diabetes are justified by many reports of elevated markers of oxidative stress in patients (Robertson et al., 2003).

\textbf{Quercetin \(10\)}, when administered orally to STZ-diabetic rats at both 50 and 80 mg/Kg for 45 days led to a decrease in thiobarbituric acid reactive species (TBARS), whereas superoxide dismutase and catalase levels were normalized (Mahesh and Menon, 2004). In another study, quercetin was given to STZ-diabetic rats (45.3 mg/Kg; intraperitoneally) for 8 weeks and although there was no decrease in blood glucose levels, there was inhibition of oxidative stress, NFkB activation and iNOS overexpression in the liver of STZ-diabetic animals. Quercetin treatment, by abolishing the IKK/NFkB signal transduction pathway (responsible for cellular response to inflammation), may block the production of noxious mediators involved in the development of early injury and in the evolution of late complications in different tissues affected by chronic hyperglycemia (Dias et al., 2005).

\textbf{Rutin \(30\)}, a quercetin glycoside, when given orally at 25-100 mg/Kg for 45 days to STZ-diabetic rats, promoted a decrease in fasting plasma glucose levels, glucosilated haemoglobin, TBARS along with lipidic hydroperoxydes (Kamalakkannan et al., 2006) and has therefore been considered helpful in protecting pancreatic islets against exposure to STZ \textit{in vitro} due to its antioxidant activity (Esmaeili et al., 2009).
**Epicatechin** (31), when administered intraperitoneally at 30 mg/Kg (one dose prior to STZ; then 2x/day for 6 days) to STZ-induced diabetic rats, promoted maintenance of blood glucose concentrations which might be due to a protective effect of epicatechin. Also, epicatechin significantly suppressed the overproduction of NO in the pancreatic islets in a dose dependent manner. Therefore, the protective effects are likely to be attributable to the suppression of NO generation in islets (Kim et al., 2003).

**Pterostilbene** (42), when given orally at 40 mg/Kg for 6 weeks to STZ-Nicotinamide-induced diabetic rats, induced antioxidant enzymes levels to normal levels (Amarnath et al., 2006). Another stilbene, **resveratrol** (57), when administered orally at 10mg/Kg for 2 weeks to STZ-induced diabetic rats, promoted a significant improvement on the levels of the renal oxidative stress markers malonaldehyde and glutathione and antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) (Sharma et al., 2006).

Finally, **ferulic acid** (4-hydroxy-3-methoxycinnamic acid), a highly abundant phenolic phytochemical present in cell walls of many plants, may have significant health benefits through its antioxidant, anticancer properties and blood glucose lowering activity (Ohnishia et al., 2004). Particularly, it has been observed that when administered orally (10-40 mg/Kg for 45days) to STZ-induced diabetic rats, decreased blood glucose levels and promoted reduction in TBARS, hydroperoxides and free fatty acids (FFA) levels in the liver. An increase of activities of antioxidant enzyme systems: SOD, CAT, Glutathione peroxidase (GPx) as well as an expansion of pancreatic islets was observed (Balasubashini et al., 2004).

A summary of the reviewed polyphenols, grouped by their corresponding antidiabetic activity, is presented in Table 2.2.
Table 2.2. Summary of polyphenols with antidiabetic activity

<table>
<thead>
<tr>
<th>Antidiabetic effect</th>
<th>Compound</th>
<th>Chemical group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose transport and absorption inhibition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin (10)</td>
<td>Flavonol</td>
</tr>
<tr>
<td></td>
<td>Isoquercitrin (11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myricetin (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epicatechin gallate (13)</td>
<td>Flavanol</td>
</tr>
<tr>
<td></td>
<td>EGCG (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epigallocatechin (15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gallocatechin (16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Luteolin (17)</td>
<td>Flavone</td>
</tr>
<tr>
<td></td>
<td>Luteolin-7-O-glucosides (18 e 22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scutellarein (19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scutellarein-7-O-glucosides (20 e 21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sissoitrin (23)</td>
<td>Isoflavone</td>
</tr>
<tr>
<td></td>
<td>Phlorizin (24)</td>
<td>Dehydrochalcone</td>
</tr>
<tr>
<td></td>
<td>Naringenin (25)</td>
<td>Flavanone</td>
</tr>
<tr>
<td></td>
<td>Cafeic acid (26)</td>
<td>Phenylpropanoid</td>
</tr>
<tr>
<td></td>
<td>Ferulic acid (27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorogenic acid (28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tannic acid (29)</td>
<td>Phenolic acid</td>
</tr>
<tr>
<td><strong>Insulin secretagogue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin (10)</td>
<td>Flavonol</td>
</tr>
<tr>
<td></td>
<td>Rutin (30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epicatechin (31)</td>
<td>Flavanol</td>
</tr>
<tr>
<td></td>
<td>Catechin (32)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Afzelechin (33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apigenin-C-glycoside (34)</td>
<td>Flavone</td>
</tr>
<tr>
<td></td>
<td>Isovitexin (35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swertisin (36)</td>
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</tr>
<tr>
<td></td>
<td>Genistein (37)</td>
<td>Isoflavone</td>
</tr>
<tr>
<td></td>
<td>Cyanidin-3-glucoside (38)</td>
<td>Anthocyanin/</td>
</tr>
<tr>
<td></td>
<td>Delphinidin-3-glucoside (39)</td>
<td>Anthocyanidin</td>
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<tr>
<td></td>
<td>Pelargonidin (40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leucopelargonidin-glycoside (41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pterostilbene (42)</td>
<td>Stilbene</td>
</tr>
<tr>
<td></td>
<td>Hydroxybenzoic acid (43)</td>
<td>Phenylpropanoid</td>
</tr>
<tr>
<td><strong>Insulin sensitizer/Insulin mimetic</strong></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin (10)</td>
<td>Flavonol</td>
</tr>
<tr>
<td></td>
<td>Kaempferol (44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kaempferitrin (45)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kaempferol-3-neohesperoside (46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myricetin (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Catechin (32)</td>
<td>Flavanol</td>
</tr>
<tr>
<td></td>
<td>EGCG (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chrysin (47)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apigenin (48)</td>
<td>Flavone</td>
</tr>
<tr>
<td></td>
<td>Apigenin-6-C-glucoside (34)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhoifolin (49)</td>
<td></td>
</tr>
</tbody>
</table>
The structures of the polyphenols referred above are now presented following the same order as they were described in the previous literature review.
Introduction
Introduction

Scutellarein (19)

Scutellarein-7-O-glucoside (20)

Scutellarein-7-O-(6-O-feruyl)-glucoside (21)

Luteolin-7-O-(6-O-feruyl)-glucoside (22)

Sissotrin (23)
Phlorizin (24)

Naringenin (25)

Caffeic acid (26)

Ferulic acid (27)

Chlorogenic acid (28)

Tannic acid (29)

Rutin (30)

Epicatechin (31)

Catechin (32)
Afzelechin (33)

Apigenin-6-C-(2′″-O-rhamnosyl)-fucoside (34)

Isovitexin (35)

Swertisin (36)

Genistein (37)

Cyanidin-3-O-glucoside (38)

Delphinidin-3-O-glucoside (39)

Pelargonidin (40)
Dimethoxy ether of leucopelargonidin-3-O-rhamnoside (41)

Pterostilbene (42)

Hydroxybenzoic acid (43)

Kaempferol (44)

Kaempferitrin (45)

Kaempferol-3-neohesperidoside (46)

Chrysin (47)

Apigenin (48)
Introduction

Rhoifolin (49)

Cosmosiin (50)

Daidzein (51)

Puerarin (52)

Daidzin (53)

4-hydroxyderricin (54)

Xantoangelol (55)

Sakuranetin (56)
Introduction

Resveratrol (57)

Dihydroeugenol (58)

Glyasperin D (59)

Dehydroglyasperin C (60)

Dehydroglyasperin D (61)

Glycryrin (62)

Glycycoumarin (63)

Mangiferin (64)

Mangiferin-7-O-glucoside (65)
Introduction

Sulfuretin (66)

Sylmarin (67)

Guaijaverin (68)

Desmanthin-1 (69)

Isorhamnetin-3-O-glucoside (70)

Isoaffnetin (71)

Butein (72)

Myrciatrin IV (73)
Considering this literature review and the summary presented in Table 2.2 it is possible to observe that there are multiple beneficial activities in which polyphenols are involved regarding carbohydrate metabolism regulation. They do so through various mechanisms:

i) inhibiting enzymes and glucose transporters,
ii) preventing and restoring integrity and function of beta-cells,
iii) promoting insulin release,
iv) improving glucose uptake and utilization and,
v) simply acting as antioxidants.

Therefore, polyphenols offer an exciting opportunity to develop novel therapeutics.

Several compounds have shown different antidiabetic activities, namely flavonol quercetin, isoflavone genistein and stilbene resveratrol have been shown to interact with most of the antidiabetic targets reviewed. Many present two and three different types of antidiabetic action like flavonols myricitin and rutin, Moreover, flavone apigenin glucoside has simultaneously presented insulin secretagogue and insulin mimetic activity.

What can be deduced from this review is that there is no clear correlation between polyphenolic structure and each specific antidiabetic activity, since classes of flavonols, flavanols, flavones, isoflavones, chalcones and stilbenes are well represented in each category of antidiabetic activity. Therefore, more detailed studies are needed in order to
look into the interactions of polyphenols and enzymes such as alpha glucosidases, aldose reductase and glucose transporters such as S-GLUT-1, GLUT-5, GLUT-4 as well as looking further down into interactions at the molecular level, biochemical pathways and gene expression.

Moreover, it is known that flavonoid-rich extracts effects are not only due to one molecule but due to synergic effects, which makes it difficult to identify the bioactive molecules (Pedrielli, and Skibsted, 2002). More work is therefore needed in order to better define the effects of each structure.


2.6.1. Botanical aspects of *Coreopsis tinctoria*

*Coreopsis tinctoria* Nutt. belongs to the Asteraceae/Compositae family and was first described in North America by Thomas Nuttall in 1823. It consists of a small, glabrous, aromatic, annual plant found in the United States in low wet areas, mostly in the Midwest, South, and mid-Atlantic Coast states, although nowadays is distributed worldwide.

![Figure 2.15](https://www.davesgarden.com)

*Figure 2.15.* General aspect of *Coreopsis tinctoria* and a detail of the flowering tops. (Drawing from Barton, 1823; Picture from www.davesgarden.com)
Its commonly bicolored ligules (yellow towards the tip, red-brown at the base) form distinctive color patterns when the populations bloom in midsummer (Figure 2.15). *Coreopsis tinctoria* has been, in years past, a favorite garden species, and still is available in several cultivars from seed companies under the old name, "Calliopsis." Several horticultural binomials (*Coreopsis radiata* Hort., *C. nigra* Hort., *C. elegans* Hort., *Calliopsis marmorata* Hort.), of no scientific standing, have been applied to it. Common names for this species include: plains coreopsis, golden tickseed, dwarf radiata. (Smith and Parker, 1971) and Estrelas-do-Egipto in Portugal (D’Oliveira Feijão, 1973).

### 2.6.2. Phytochemical review for *Coreopsis tinctoria* flowering tops

The genus *Coreopsis* possesses a quite variable flower phytochemical pattern that includes mainly chalcone-aurone pairs, namely butein derivatives (67, 72, 76, 77) and okanin derivatives (78, 79 and 80) (Figure 2.16) which are very common in *C.maritima*, *C.gigantea*, *C.bigelovii*, *C.grandiflora*, *C.mutica*, *C.tinctoria* and, lanceolatin derivatives (81, 82, 83 and 84) (Figure 2.16) which have also been found in *C.grandiflora*, *C.lanceolata* and *C.saxicola* (Crawford and Smith, 1980; Nicholls and Bohm, 1979; Crawford, 1970; Geissman et al., 1956; Harborne and Geissman, 1956; Shimokoriyama and Hatori, 1953; Geissman and Heaton, 1944; Geissman and Heaton, 1943).
### Butein derivatives

<table>
<thead>
<tr>
<th>Structure</th>
<th>R = glucose: Coreopsin (76)</th>
<th>R = glucose: Sulfurein (77)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Butein Derivative 1" /></td>
<td><img src="image2" alt="Butein Derivative 2" /></td>
<td></td>
</tr>
<tr>
<td>R = H: Butein (72)</td>
<td>R = H: Sulfuretin (67)</td>
<td></td>
</tr>
</tbody>
</table>

### Okanin derivatives

<table>
<thead>
<tr>
<th>Structure</th>
<th>R = glucose: Marein (78)</th>
<th>R = glucose: Maritimein (80)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Okanin Derivative 1" /></td>
<td><img src="image4" alt="Okanin Derivative 2" /></td>
<td></td>
</tr>
<tr>
<td>R = H: Okanin (79)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Lanceolatin derivatives

<table>
<thead>
<tr>
<th>Structure</th>
<th>R = glucose: Lanceolin (81)</th>
<th>R = glucose: Leptosin (83)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5" alt="Lanceolatin Derivative 1" /></td>
<td><img src="image6" alt="Lanceolatin Derivative 2" /></td>
<td></td>
</tr>
<tr>
<td>R = H: Lanceolatin (82)</td>
<td>R = H: Leptosidin (84)</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.16.** Floral phytochemicals from the genus *Coreopsis*. Butein, okanin, lanceolatin chalcone-aurore derivatives.
Coreopsis tinctoria phytochemistry has been studied for the first time during the 1950’s by Shimokoriyama, who reported two anthochlor glycosides isolated from an ethanolic flower extract. These compounds were regarded as major constituents in the yellow coloured part. One of them had the properties of a chalcone and the other those of a benzalcoumaranone. The first was identified as marein (okanin-4’-O-glucoside) (78) and the later, maritimein (3’,4’,7-trihydroxyaurone-6-O-glucoside) (80). The author has also reported that the chalcone glycoside had great tendency to isomerise during the course of isolation, into the colourless flavanone glycoside which he identified as flavanomarein (85) (Figure 2.17) (Shimokoriyama, 1957). Okanin (79), marein’s aglycone, was identified as well in this species (Shimokoriyama, 1957; Geissman et al., 1956) and so did coreopsin (butein-4’-O-glucoside) (76) (Harborne, 1977) which can also be found in closely related genera such as Pyrrhopappus (Harborne, 1977) Cosmos (Shimokoriyama and Hattori, 1953) and Bidens (Geissman et al., 1956).

Beside chalcones, aurones and flavanones, other types of flavonoids have been described for C. tinctoria, like the flavone luteolin (17), which has been identified by Puri and Seshadri in 1954 and the anthocyanin chrysanthemin (cyanidin-3-O-glucoside) (38) found in the red part of the upper epidermis of the petals and identified by Hayashi and Abe in 1953. Other polyphenols present include phenylpropanoic acids such as caffeic acid (26) and chlorogenic acid (28) (Figure 2.17) (Shimokoriyama, 1957).

More recently, another study of the methanolic extract of Coreopsis tinctoria flowers (Zhang et al., 2006) confirmed the presence of the previous compounds or reported for the first time the presence of the known flavonoids. They identified flavanomarein (85), marein (78) (Hoffmann and Hoelzl, 1989), quercetagetin-7-O-glucoside (86) (Ramachandran Nair et al., 1995), (2R, 3R)-dihydroquercetin-7-O-glucoside (87) (Hefeng and Lennart, 1996), okanin (79), quercetin (10), butein (72) (Shimokoriyama and Hattori, 1953; Geissman et al., 1956), 2S-3’,4’,7,8-tetrahydroxyflavanone (88) (Foo, 1987), (2R, 3R)-3,3’,5,5’,7-pentahydroxyflavanone (89) (Ding et al., 1997), (2R, 3R)-3,4’,5,6,7-pentahydroxyflavanone (90) (Piccinelli et al., 2004) and 2S-3’,5,5’,7-tetrahydroxyflavanone (91) (Yi et al., 2002), and the new compound: okanin-4’-O-(6”-O-malonyl)-glucoside (92) (Figure 2.17).
### Introduction

<table>
<thead>
<tr>
<th>Chalcones</th>
<th>Aurones</th>
</tr>
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<tbody>
<tr>
<td><img src="image" alt="Butein (72)" /></td>
<td><img src="image" alt="Maritimein (80)" /></td>
</tr>
<tr>
<td><img src="image" alt="Coreopsin (76)" /></td>
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<tr>
<td><img src="image" alt="Marein (78)" /></td>
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</tr>
<tr>
<td><img src="image" alt="Okanin (79)" /></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Okanin-4’-O-(6’’-O-malonyl)-glucoside (92)" /></td>
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</tbody>
</table>

89
<table>
<thead>
<tr>
<th>Flavanones</th>
<th>Flavonols</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Flavanomarein" /></td>
<td><img src="image" alt="Quercetin" /></td>
</tr>
<tr>
<td><img src="image" alt="2R, 3R-3',5',7-pentahydroxyflavanone" /></td>
<td><img src="image" alt="2R, 3R-dihydroquercetin-7-O-glucoside" /></td>
</tr>
<tr>
<td><img src="image" alt="2S-3',4',7,8-tetrahydroxyflavanone" /></td>
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</tr>
<tr>
<td><img src="image" alt="2S-3',5',7-tetrahydroxyflavanone" /></td>
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</table>

**Introduction**
Introduction

<table>
<thead>
<tr>
<th>Anthocyanins</th>
<th>Chrysanthemin (cyanidin-3-O-glucoside) (38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl-</td>
<td>Caffeic acid (26)</td>
</tr>
<tr>
<td>propanoids</td>
<td>Chlorogenic acid (28)</td>
</tr>
</tbody>
</table>

**Figure 2.17.** *C. tinctoria* floral phytochemicals grouped by type of flavonoid structure.

### 2.6.3. Traditional use worldwide and in Portugal

*Coreopsis tinctoria* is native from North America where native Indians have used them to treat several disorders including diarrhoea, internal pains, bleeding, to strengthen blood and as an emetic (Foster and Duke, 1990). There is also documented use of the plant in Traditional Chinese Medicine where a decoction of the whole plant is used for diarrhoea (Cui and Ran, 1993). *Coreopsis tinctoria’s* use has been referred in a traditional Chinese formula for diabetes ("Qijú Liùwèi wán" currently known as "Qijú Dìhuáng wán") as early as during the Yuan Dinasty (1271-1368 AD) by Huá Shòu. Additionally, in Portugal, two cups per day of an infusion of *C. tinctoria* flowering tops has been traditionally used to control diabetes (D’Oliveira Feijão, 1973).