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Alpha Amylase inhibitory plants

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Abstract:

Alpha-amylase is a low molecular weight endohydrolase enzyme that catalyzes the hydrolysis of starch and, ultimately, the production of glucose. This enzyme is considered one of the key enzymes and its inhibition can play a significant role in the treatment of diabetes. By controlling the catalytic activity of this enzyme, we can expect a reduction in glucose production in the post-meal phase, which can be a therapeutic advantage for people with diabetes.

Today, the importance of using natural sources as inhibitors of alpha-amylase enzyme compared to chemical inhibitors has been much considered due to fewer side effects. Plant's inhibitory effects on this enzyme can be attributed to their secondary metabolites, such as phenolics, terpenoids, flavonoids and coumarin phytochemicals. Some plant extracts, especially extracts rich in proanthocyanidin, can inhibit enzymes such as alpha-amylase, which can help control and treat diabetes.

Key words: Alpha-amylase, Inhibition, Herbal extract, Diabetes.

1. Introduction

Enzymes are protein molecules and biological catalysts needed in all living organisms to carry out biological reactions. These catalysts speed up various reactions by lowering the activation energy. Enzymes are sensitive to different physical and chemical conditions, which can have positive or negative effects on their performance and even cause enzyme inactivation [1].

Alpha amylase is one of the most widely used commercial enzymes. This enzyme creates smaller sugars, such as glucose and maltose, by breaking α-1→4 glycosidic bonds in starch or other polysaccharides. Although most alpha-amylases are secreted extracellularly, some intracellular alpha-amylases have been reported so far. A wide range of organisms, including aquatic bacteria, fungi, actinomycetes, animals and plants, can produce alpha-amylase [2].

Starch is known as the main source of energy in the human diet. Food sugars and starches are broken down into glucose by amylase enzymes. Research has observed a relationship between pancreatic alpha-amylase activity and glucose levels after eating. Alpha-amylase is a metalloenzyme that helps to increase blood glucose levels and postprandial hyperglycemia by breaking down polysaccharides into smaller molecules. As a result, it is recommended to produce inhibitors that can reduce the release of glucose from carbohydrate chains or prolong its absorption and ultimately reduce plasma glucose level after a meal. According to phytochemical research, bioactive compounds with inhibitory properties have been identified in various plant extracts. Despite the availability of antidiabetic drugs, herbal treatments can be used as a suitable drug alternative in the control and treatment of diabetes due to fewer side effects and cost-effectiveness [3].

Secondary metabolites produced by plants have various biological properties, and many of them can be used as medicine [4].

Medicinal plants are an important source of phytochemical compounds with the potential to inhibit alpha-amylase and can play a significant role in controlling diabetes. This inhibitory effect on alpha-amylase may be due to alkaloids, flavonoids, saponins, tannins and sterols, which have been observed by phytochemical screening [5].

Research has discovered a correlation between alpha-amylase inhibition or antioxidant capacity and total phenolic or flavonoid content. The results suggest that the traditional use of certain plant species in the treatment of diabetes can be beneficial [6].

2. Research methodology

The present study was conducted in a review method using the keywords alpha-amylase, inhibitory properties, plant extracts, secondary metabolites, diabetes and the title of the article between the years 2000 and 2024 using Google Scholar, ScienceDirect, PubMed and Google databases.

3. Findings

Acording to research, starch hydrolase inhibitors from cereals have been reported for phenolic acids, tannins, anthocyanins and flavonoids. So far, enzyme inhibition has been investigated by various methods, and the results have shown that the compounds may be the main enzyme inhibitors in cereals. Also, phenolic compounds are mainly concentrated in pericarp, skin bran and aleurone layers of whole grains, which may increase their contribution to enzyme inhibitory activity. Phenolic extracts of these fractions usually show strong inhibitory activity towards α-glucosidase and α-amylase compared to other parts of whole grains [7].

Researchers have tested the use of medicinal plants as alpha-amylase inhibitors, and it has been reported that about 800 different plant species have shown anti-diabetic properties in blood. So far, a list of plants has been reported that have significant inhibitory activity against the α-amylase enzyme. A summary of these plants is given in the table below [8].

Table 1: Plants with α-Amylase inhibitory effect [8].

Plant	**Part used**	**Type of extract**	**Activity (% inhibition concentration) (mg/ml)**	**Control**	**References**
Acanthaceae Andrographis paniculate Nees	Leaf and aerial parts	Ethanol	52.5 (50.9) 54.8 (11.3)	Acarbose with 50.1% of maxim inhibition at 10mg/mL	Rammohan Subramanian et al. Acta Biochim Pol. 2008.
Actinidiaceae Actinidia deliciosa	Leaf	Methanol 90%	50 (0.0429)	Voglibose with 50% of inhibition at 0.0466mg/mL	Miyuki Shirosaki et al. Biosci Biotechnol Biochem. 2008 Apr.
Balanitaceae Balanitesa egyptiaca L	Bark	Aqueous buffered	45-75(200)	Acarbose inhibition higher than 75% at 200 mg/mL	Ingrid Funke, Matthias F. Melzig ; Germany,Mar 2006
Coniferae Ginkgo biloba L	Leaf	Ethanol	70 (50)	Non-treated enzyme	Marcia Da Silva Pinto et al. Bioresour Technol. 2009 Dec.
Ericaceae Vaccinium myrtillus L.	Leaf	Aqueous buffered	>75 (200)	Acarbose, inhibition higher than 75% at 200 mg/mL	Ingrid Funke, Matthias F. Melzig ; Germany,Mar 2006
Geraniaceae Geranium pratense L.	Aerial part	Methanol	43.9 (0.3mg/mL)	Acarbose with 79.6% of inhibition at 0.1 mg/mL	Kyoko Kobayashi et al. Biol Pharm Bull. 2003 Jul.

Fabaceae Cajanus cajan L.	Seed	Aqueous buffered	100 (2mg protein)	Non-treated enzyme	Syed Bilal Shah et al. Pak J Pharm Sci. 2018 Jul.
Malvaceae Hibiscus sabdariffa Linn.	Flower	Methanol	50% 100 (10mL/g fr. wt.)	Non-treated enzyme	Lili Kandra et al. Biochem Biophys Res Commun. 2004.
Myrsinaceae Embelia ribes Burm. f.	Seed	Ethanol	59.3	Phaseolus vulgaris with 59.4% of inhibition at 0.0125 mg/mL	D Prashanth et al. Fitoterapia. 2001 Feb.
Paeoniaceae Paeonia anomala L.	Root	Methanol	33.1 (0.3mg/mL)	Acarbose with 79.6% of inhibition at 0.1 mg/mL	Kyoko Kobayashi et al. Biol Pharm Bull. 2003 Jul.
Pinaceae Cedrus libani A. Rich	Essential oils from cones	Aqueous buffered	31 (1)	Acarbose with 50 % of at inhibition 1.22 mg/mL	Monica R Loizzo et al. J Ethnopharmacol. 2008.
Polygalaceae Securidaca longepidunculata Fresen	Root	Aqueous buffered	20-45 (200mg/mL)	Acarbose with inhibition higher than 75% at 200 mg/mL	Ingrid Funke, Matthias F. Melzig ; Germany,Mar 2006
Punicaceae Punica granatum L.	Fruit rind	Ethanol	68.2 (1)	Phaseolus vulgaris with 59.4% of inhibition at 0.0125 mg/mL	D Prashanth et al. Fitoterapia. 2001 Feb.
Rosaceae Pentaphylloides fruticosa (L.)	Leaf and branch	Methanol	31.2 (0.3mg/mL)	Acarbose with 79.6% of inhibition at 0.1 mg/mL	Kyoko Kobayashi et al. Biol Pharm Bull. 2003 Jul.
Rubiaceae Mitragyna inermis (Wild)	Leaf	Aqueous buffered	75	Acarbose with inhibition higher than 75% at 200 mg/mL	Ingrid Funke, Matthias F. Melzig ; Germany,Mar 2006
Rutaceae Murraya koenigii L.	Leaf	Chloroform	56.64	Acarbose with 50 % of at inhibition 1.22 mg/mL	Menakshi Bhat et al. Evid Based Complement Alternat Med. 2011.
Saxifragaceae Bergenia ciliata, Haw.	Rhizome	Methanol 50%	93.5 (150)	Non-treated enzyme	BoMi Ryu Bioorganic & Medicinal Chemistry, 2009
Theaceae Camellia sinensis L.	Leaf	Aqueous buffered	45-75(200)	Acarbose with inhibition	Ingrid Funke, Matthias F.

				higher than 75% at 200 mg/mL	Melzig ; Germany,Mar 2006

In another study conducted by Tahereh Eghbali Zarch and colleagues in 2010 in Iran; the seeds of *Silybum marianum* were collected, and after preparing different extracts with different solvents, the inhibitory effect on alpha-amylase and alpha-glucosidase was measured [9].

Table 2: Inhibitory values of the total extract and different fractions of Silybum marianum seeds in terms of mean and standard deviation. *: Has a significant difference compared acarbose ($p<0.05$); **: Has a significant difference compared acarbose ($p<0.01$); ***: Has a significant difference compared acarbose ($p<0.001$) [9].

Extract name	**% α-Amylase inhibition**	**Extract concentration (µg/kg)**	**IC50 (µg/kg)**
Total Methanolic	35.4±2.3 51.2±1.4 61.4±2.1 84.7±2.2	25 50 75 100	43.1±0.7*
Hexan	8.8±1.5 22.6±2.8 30.3±4.1 41.7±3.5	25 50 75 100	162.43±8.93***
Chloroform	3.6±1.3 21.1±2.5 30.3±2.2 33.5±0.9	25 50 75 100	195.57±12.36***
Ethyl acetate	10.1±1.7 42.5±2.3 52.0±4.3 68.6±3.6	25 50 75 100	65.5±0.7***
Methanol	21.8±2.2 39.1±3.3 61.0±3.1 78.2±2.3	25 50 75 100	55.3±2.6**
Acarbose	30.1±1.9 40.2±2.3 62.4±1.9 79.7±3.7	10 20 40 80	24.9±2.0

4. Discussion

One of the most important health challenges in the 21st century is diabetes mellitus. This disease can be associated with many side effects, such as cardiovascular diseases, retinopathy, neuropathy, nephropathy and liver disorders. In order to control chronic hyperglycemia, the development of potential inhibitors of alpha-amylase and alpha-glucosidase is considered an important strategy [10].

Amylase enzyme is a calcium-dependent metalloenzyme whose molecular weight range is usually between 54 and 62 kDa. Amylase is mainly secreted by the pancreas and salivary glands but is also present in other tissues at minimal levels [11]. This enzyme was originally called diastase, but it was renamed "amylase" in the early 20th century. The main role of amylases is to break the glycosidic bonds within starch molecules and generally convert complex carbohydrates into simpler sugars. Amylases are divided into 3 main classes: alpha, beta and gamma amylases [12]. Each of them targets specific parts of the carbohydrate molecule. Alpha-amylase in humans, animals, plants and microbes, beta-amylase primarily in microbes and plants, and gamma-amylase can also exist in both animals and plants [13].

α-Amylase has 3 domains A, B and C. Domain A is the largest domain that presents a typical barrel superstructure (β/α)8. The B domain is located between the A and C domains and is connected to the A domain by a disulfide bond. The C domain has a β-sheet structure linked to the A domain by a simple polypeptide chain and appears to be an independent domain of unknown function. The active site (substrate binding) of α-amylase is located in a long cleft between the carboxyl terminal of the A and B domains. Calcium (Ca2+) is located between domains A and B and seems to play a role in stabilizing the three-dimensional structure of the enzyme and also as an allosteric activator. Asp206, Glu230, and Asp297 participate in catalysis according to the binding of substrate analogs [14]. The binding site to the substrate consists of 5 sub-sites; the catalytic site is located in the 3rd sub-site. Substrate can bind to the first glucose residue in subsite 1 or 2, allowing cleavage to occur between the first and second or second and third glucose residues [15].

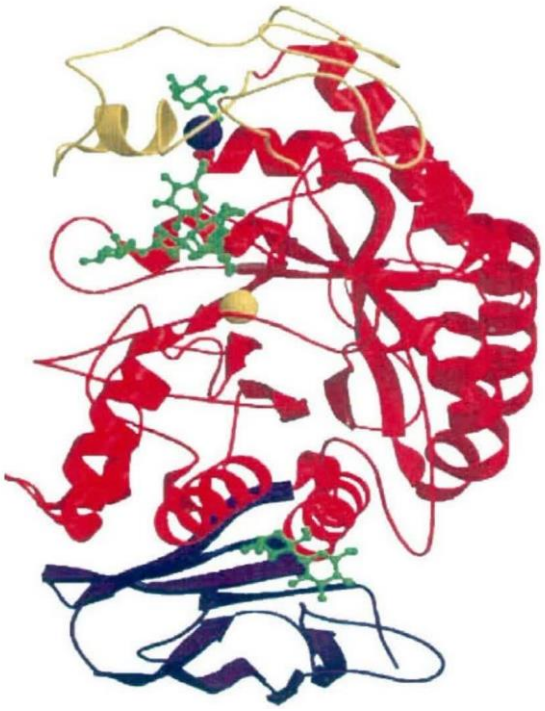

Figure 1: α-Amylase structure. Domain A is shown in red, domain B in yellow and domain C in purple. Calcium ion is in the form of a blue sphere and chloride ion is in the form of a yellow sphere in the catalytic center. The green structure are bound to the active site and to the surface binding sites [16].

In the digestive tract, complex carbohydrates are converted into monosaccharides by several breakdown reactions, which are absorbed in the small intestine. The digestion process begins with the secretion of amylases, which are mainly produced by the pancreas and salivary glands, and catalyze the hydrolysis of starch into short polysaccharides[17]. The acidic environment of the stomach prevents the enzyme activity of salivary amylase, which prevents the further breakdown of starch. After entering the small intestine, starch is digested by pancreatic amylases [18]. The final step in carbohydrate metabolism is carried out by alpha-glucosidases in the brush border of enterocytes. Polysaccharides and monosaccharides resulting from the action of alpha-amylase and alpha-glucosidase are absorbed by the body at different speeds. Therefore, inhibiting the activity of alpha-amylase and alpha-glucosidase can delay the release of glucose from complex carbohydrates and control postprandial hyperglycemia. As a result, this problem is considered an ideal target for diabetes management [19].

According to the research, the antidiabetic effect of simple phenolic acids such as gallic, protocatechuic, ellagic, syringic or salicylic acids has been investigated due to their role in the function of glucose and insulin receptors. Extracts of different fruits and vegetables have been

investigated for their antidiabetic potential. In most of these studies, raw or pure extracts have been used, and their inhibitory activity has been attributed to polyphenol compounds. Polyphenols are a large and heterogeneous group of phytochemicals found in plants and an essential part of the human diet. Phenolic acids are one of the most common polyphenols, which consist of aromatic phenols from plant secondary metabolites with a functional carboxylic acid group. One hypothesis is that their inhibitory activity towards α-amylase and α-glucosidase enzymes is related to their structure, which allows them to interact with the enzyme or reaction substrate [20].

Flavonoids are divided into different groups depending on the substitution in the heterocyclic ring (ring C). Some of these subgroups include flavanones (such as hespertin), flavones (luteolin), flavonols (quercetin), and flavanols (catechin). Flavonoids usually exist in a glycosylated form by attaching a sugar moiety (such as rutin) [21].

Plant flavonoids have the potential to inhibit starch digestive enzymes due to their ability to bind non-covalently to the active site residues of enzymes [22].

Preparing medicinal plants for experiments is the first step and a key point to achieve quality results from research. This important step includes extracting and determining the quality and quantity of bioactive compounds [23].

Solvents have a significant effect on the amount and nature of secondary metabolites of medicinal plants, and this issue is important in the extraction process. Several studies have reported the effect of solvents on various secondary metabolites or their biological properties [24].

Today, herbal extracts can be used for combined treatments along with standard drugs[25].

Due to their natural origin, plants are excellent candidates for replacing artificial compounds that may have negative effects are considered Extracting these compounds from natural sources and determining their activity in products is an important challenge in the direction of producing products with positive effects on human health [26].

5. Conclusion

Alpha-amylase is considered a key enzyme in the hydrolysis of starch, and nowadays artificial inhibitors such as acarbose are used to inhibit it. Considering the side effects that synthetic inhibitors can cause, finding natural herbal alternatives can be helpful. According to the research that has been done so far, and some of them are mentioned in this review article, the secondary metabolites of plants can have an inhibitory effect on the activity of alpha-amylase. According to the review of the articles mentioned in this study, the type of solvent used in extract preparation, as well as different parts of a plant, can affect the result of enzyme activity. However, further research in this field is still recommended in vitro and in vivo.

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