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Research Article

Evaluation of α-Glucosidase Inhibitory Action of Isolated Compound beta Amyrin from *Memecylon umbellatum* Burm. F

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ABSTRACT
Numerous metabolic disorders and diseases associated with the glucosidase enzyme activity. Therefore, in the present study we evaluated alpha glucosidase inhibitory effect of isolated bioactive compound of *Memecylon umbellatum*. The isolated bioactive compound screened for α-glucosidase inhibitory activity using yeast glucosidase. The enzyme kinetics studied Line weaver Burk plot method. The α-glucosidase enzyme kinetics effect measured using the method slightly modified from (Dahlqvist, 1964.). The result shows that isolated bioactive compound Beta Amyrin of Memecylon umbellatum leaves were non toxic and shows significant anti-diabetes activity.

Keywords: α-glucosidase Inhibition, Memecylon umbellatum, Iron wood tree, Lineweaver Burk, Beta Amyrin, Bioactive compounds, Anti-diabetes activity

INTRODUCTION

Memecylon umbellatum is a shrub or a small tree belonging to the family Melastomataceae. In Ayurveda, the leaves are used as a cooling astringent used to treat eye trouble. The leaves are reported to possess antiviral activity (Kirthikar K.R *et al*). The leaves were also used in snake bite (Kshirsagar R.D and Singh N.P). The leaf powder is used for the treatment of Diabetes (Ayyanar, M.K.Sankarasivaraman, S.Ignacimuthu). The genus Memecylon L. comprise of about 300 species in the world, of which 30 species has been reported from India (Santapaus and Henry *et al*., 1989) and 16 species from Tamil Nadu (Nair and Henry, 1983). *Memecylon umbellatum Burm* (Family Melastomataceae) is small evergreen shrub or tree having young tree branches and bears numerous cymes. The plant is known as "*Anjani*" in Sanskrit and "*Iron wood tree*" in English. *Memecylon umbellatum* is an ethno-medicinal plant used traditionally for treating various diseases. Ethno-medicinally, leaves are used to treat eye troubles; gonorrhea, leucorrhea, and wounds (Dhar *et al.*, 1968, Puratchikodi and Nagalakshmi, 2007). M.umbellatum possesses remarkable antioxidant and anti-inflammatory potential. Isolation of various phyto-constituents like β-Amyrin, Sitosterol, Oleanolic acid, Ursolic acids, Sitosterol-β-D-glucosidase and a new substance umbelactone from the aerial part of *Memecylon umbellatum* have been reported (Agarwal. S.K, Rastogi R.P). Terpenoids are synthesized from acetate units and as such they share their origin with fatty acids. The Triterpenoids are a large and derived from a C_{30} acyclic compound squalene or a related precursor. Plant terpenoids are used extensively for their aromatic qualities.

The early stage of diabetes mellitus type2 is associated with post prandial hyperglycemia due to impaired -after meal acute insulin secretion. Hyperglycermia is believed to increase the production of free radicals and reactive oxygen species, leading to oxidative tissue damage and diabetic complication such as nephropathy, neuropathy, retinopathy and memory impairment (Maritim AC, Sanders RA, Watkins JB). In Diabetes mellitus the insulin- sensitive peripheral tissues, glucose uptake and metabolism in response to insulin get reduced. In the pathogenesis of peripheral insulin resistance defective glucose transport system may perform the significant role. Balancing glucose homeostasis and clearing post prandial glucose burden is a very important step in target tissue (Shulman GL, 2000). One of the available glucose-lowering treatments is alpha-Glucosidase inhibitors (AGIs). The alpha-glucosidase enzyme is necessary for the breakdown of carbohydrates to absorbable monosaccharides which are located in the brush border of a small intestine. The (AGIs) help in delaying but do not inhibit the adsorption of consumed carbohydrates reducing the post prandial glucose and insulin peaks (Stuart AR, Gulve EA, Wang M,). Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, protein, fat, Electrolytes and water it includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar level are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin

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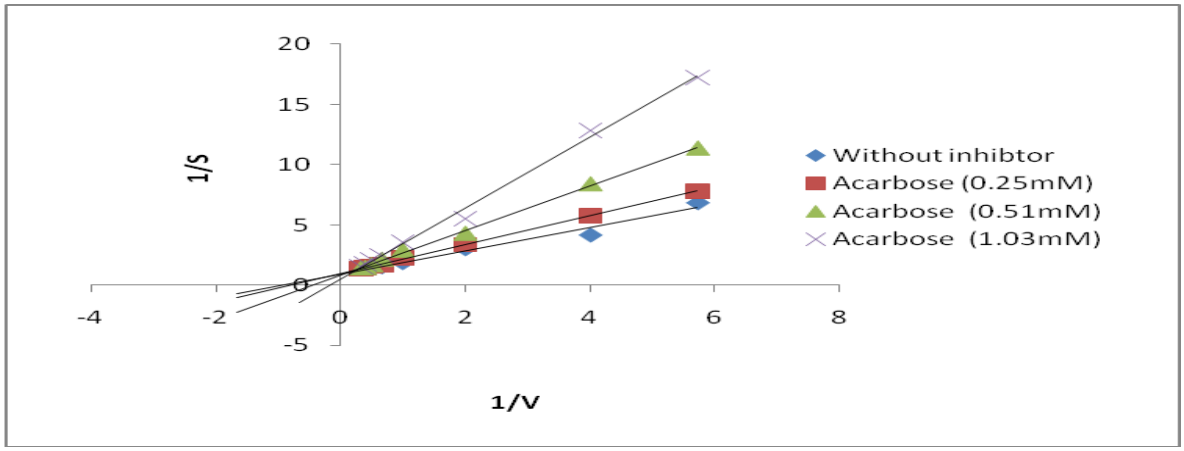

Figure 1: Shows Lineweaver-Burk plot of α- Glucosidase and Acarbose

(West IC, 2000). Therefore a therapeutic approach to treat diabetes is to decrease post prandial hyperglycemia (Chakrabarti R, & Rajagopalan R, 2002).This can be achieved by the inhibition of carbohydrate hydrolyzing enzyme like alpha glucosidase (Bhosale U.P and Hallale B.V).

Glucosidases are a group of digestive enzyme which breakdown the dietary carbohydrates into simple monosaccharides. Glucosidase inhibitors such as acarbose reduce the rate of carbohydrate adsorption from the digestive tract therefore, they have a potential to prevent the development of type2 diabetes mellitus by lowering the after-meal glucose level (Liu L, Deseo MA, Morris C, Winter KM, Leach DN). Alpha glucosidase inhibitors are used as oral anti diabetic drugs for treating type2 diabetes mellitus. They act by preventing the digestion of carbohydrates such as starch carbohydrates are normally converted into simple sugars which can be absorbed through the intestine (Bischcoft. H, 1994). Alpha glucosidase inhibitors act as competitive inhibitors of alpha glucosidase enzyme needed to digest carbohydrates. The intestinal alpha glucosidase hydrolyze complex carbohydrates to glucose and other monosaccharides in the small intestine, Inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates (Bhat M, Zinjarde SS, Bhargava SY, Kumar AR, Joshi BN, 2011). The present study was carried out to investigate the inhibitory potential of isolated Bio- active compound of Beta amyrin of *Memecylon umbellatum* on alpha glucosidase, the key enzyme responsible for carbohydrate hydrolysis.

MATERIALS AND METHODS

Drugs and Reagents

Glucoxidase-Peroxidase kit, 4-Amino anti-pyrin, Yeast α-glucosidase, were obtained from the BioGE Health Care and Sigma Chemicals Co., USA. Acarbose used as standard, Bioactive compound Beta Amyrin. All chemicals used were of analytical reagent grade.

α-Glucosidase Inhibition of Beta-Amyrin

As larger amounts of substrate are added to a reaction, the available enzyme binding sites become filled to the limit of V_{max}. Beyond this limit the enzyme is saturated with substrate and the reaction rate ceases to increase. The reaction catalysed by an enzyme uses exactly the same reactants and produces exactly the same products as the uncatalysed reaction. Like other catalysts, enzymes do not alter the position of equilibrium between substrates and products (Wrighton, MS., & Ebbing. DD,1993). However, unlike uncatalysed chemical reactions, enzyme-catalysed reactions display saturation kinetics. For a given enzyme concentration and for relatively low substrate concentrations, the reaction rate increases linearly with substrate concentration; the enzyme molecules are largely free to catalyse the reaction, and increasing substrate concentration means an increasing rate at which the enzyme and substrate molecules encounter one another. However, at relatively high substrate concentrations, the reaction rate asymptotically approaches the theoretical maximum; the enzyme active sites are almost all occupied and the reaction rate is determined by the intrinsic turnover rate of the enzyme. The substrate concentration midway between these two limiting cases is denoted by K_M. The two most important kinetic properties of an enzyme are how quickly the enzyme becomes saturated with a particular substrate, and the maximum rate it can achieve. Knowing these properties suggests what an enzyme might do in the cell and can show how the enzyme will respond to changes in these conditions.

Enzyme Assays

Enzyme assays are laboratory enzymes procedures that measure the rate of enzyme reactions. Because are not consumed by the reactions they catalyse, enzyme assays usually follow changes in the concentration of either substrates or products to measure the rate of reaction. There are many methods of measurement. Spectrophotometric assays observe change in the absorbance of light between products and reactants; radiometric assays involve the incorporation or release of radio activity to measure the amount of product made over time. Spectrophotometric assays are most convenient since they allow the rate of the reaction to be measured continuously. Although radiometric assays require the removal and counting of samples (i.e., they are discontinuous assays) they are usually extremely sensitive and can measure very low levels of enzyme

Table 1: Shows V_{max} and K_m values of Acarbose

Michaelis-Menten	Without inhibitor	Acarbose 0.25mM	Acarbose 0.51mM	Acarbose 1.03mM
V_{max}	1.027 ± 0.05318	1.035 ± 002316.	1.253 ± 0.1263	1.603 ± 0.2073
K_m	0.9133 ± 0.1283	1.261 ± 0.06682	2.37 ± 0.4384	4.666 ± 0.8832

Table 2: Shows the V_{max} and K_m values of Beta Amyrin

Michaelis-Menten	Without inhibitor	Beta Amyrin 2.5mM	Beta Amyrin 5.1mM	Beta Amyrin 10.0mM
V_{max}	1.021 ± 0.05357	1.096 ± 0.07792	1.213 ± 0.1127	1.614 ± 0.1825
K_m	0.8951 ± 0.1285	1.176 ± 0.2038	1.899 ± 0.3528	3.588 ± 0.6432

activity. An analogous approach is to use mass spectrometry to monitor the incorporation or release of stable isotopes as substrate is converted into product.

Single-substrate Reactions

Enzymes with single-substrate mechanisms include isomerases such as triose phosphate isomerase or bisphospho glycerate mutase, intra molecular lyases such as cyclone and the hammer head ribozyme, an RNA lyase (Duggleby RG, 1995).However, some enzymes that only have a single substrate do not fall into this category of mechanisms. Catalase is an example of this, as the enzyme reacts with a first molecule of hydrogen peroxide substrate, becomes oxidised and is then reduced by a second molecule of substrate. Although a single substrate is involved, the existence of a modified enzyme intermediate means that the mechanism of catalase is actually a ping-pong mechanism, a type of mechanism that is discussed in the *Multi-substrate reactions* section below. As enzyme-catalysed reactions are saturable, their rate of catalysis does not show a linear response to increasing substrate. If the initial rate of the reaction is measured over a range of substrate concentrations (denoted as [S]), the reaction rate (v) increases as [S] increases, as shown on the right. However, as [S] gets higher, the enzyme becomes saturated with substrate and the rate reaches V_{max}, the enzyme's maximum rate.

Michaelis-Menten Kinetic Model

The Michaelis-Menten kinetic model of a single-substrate reaction is shown on the right. There is an initial bimolecular reaction between the enzyme E and substrate S to form the enzyme-substrate complex ES. Although the enzymatic mechanism for the unimolecular reaction $ES \xrightarrow{k_{cat}} E + P$ can be quite complex, there is typically one rate-determining enzymatic step that allows this reaction to be modeled as a single catalytic step with an apparent unimolecular rate constant k_{cat}. If the reaction path proceeds over one or several intermediates, k_{cat} will be a function of several elementary rate constants, whereas in the simplest case of a single elementary reaction (e.g. no intermediates) it will be identical to the elementary unimolecular rate constant k_2. The apparent unimolecular rate constant k_{cat} is also called *turnover number* and denotes the maximum number of enzymatic reactions catalysed per second (Fig. 3.1.). The Michaelis-Menton equation describes how the (initial) reaction rate v_0 depends on the position of the substrate-binding and the rate constant k_2.

$$v_0 = \frac{V_{\max}[\mathrm{S}]}{K_M + [\mathrm{S}]}$$ *(Michaelis–Menten equation)*

Linear Plots of the Michaelis-Menten Equation

Line weaver–Burk or double-reciprocal plot of kinetic data, showing the significance of the axis intercepts and gradient (Fig. 3.2).

The plot of v versus [S] above is not linear; although initially linear at low [S], it bends over to saturate at high [S]. Before the modern era of non linear curve-fitting on computers, this non linearity could make it difficult to estimate K_M and V_{max} accurately. Therefore, several researchers developed linearisations of the Michaelis-Menten equation, such as the Lineweaver–Burk plot, the Eadie-Hofstee diagram and the Hanes-Woolf plot. All of these linear representations can be useful for visualising data, but none should be used to determine kinetic parameters, as computer software is readily available that allows for more accurate determination by non linear regression methods.

The Lineweaver-Burk plot or double reciprocal plot is a common way of illustrating kinetic data. This is produced by taking the reciprocal of both sides of the Michaelis–Menten equation. As shown on the right, this is a linear form of the Michaelis–Menten equation and produces a straight line with the equation $y = mx + c$ with a y-intercept equivalent to $1/V_{max}$ and an x-intercept of the graph representing $-1/K_M$.

$$\frac{1}{v} = \frac{K_M}{V_{\max[}\mathrm{S}]} + \frac{1}{V_{\max}}$$

Naturally, no experimental values can be taken at negative 1/[S]; the lower limiting value1/[S] = 0 (the$y$-intercept) corresponds to an infinite substrate concentration, where$1/v = 1/V_{max}$ as shown at the right; thus, the x-intercept is an extrapolation of the experimental data taken at positive concentrations. More generally, the Lineweaver-Burk plot skews the importance of measurements taken at low substrate concentrations and, thus, can yield inaccurate estimates of V_{max} and K_M. (Jones ME, 1992). A more accurate linear plotting method is the Eadie-Hofstee plot. In this case, v is plotted against v/[S]. In the third common linear representation, the Hanes-Woolf plot, [S]/v is plotted against [S]. In general, data normalisation can help diminish the amount of experimental work and can increase the reliability of the output, and is suitable for both graphical and numerical analysis.

Practical Significance of Kinetic Constants

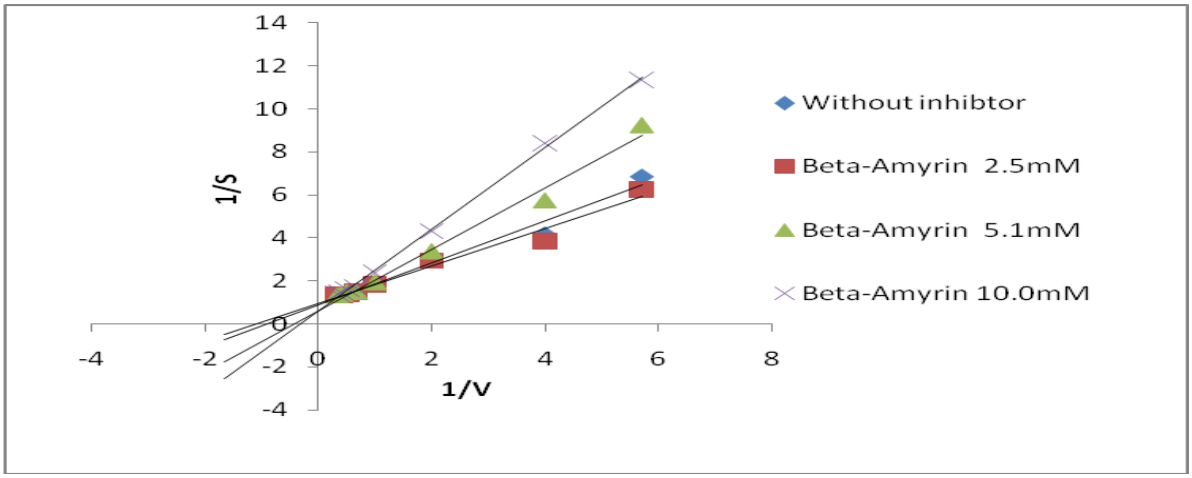

Figure 2: Shows Lineweaver–Burk plot of α-Glucosidase and Beta Amyrin

The study of enzyme kinetics is important for two basic reasons. Firstly, it helps explain how enzymes work, and secondly, it helps predict how enzymes behave in living organisms. The kinetic constants defined above, K_M and V_{max}, are critical to attempts to understand how enzymes work together to control metabolism. Making these predictions is not trivial, even for simple systems. For example, oxalo acetate is formed by malate dehydrogenase with in the mitochondrion. Oxaloacetate can then be consumed by citrate synthase, phosphoenol pyruvate carboxykinase or aspartate amino-transferase, feeding into the citric acid cycle, gluconeogenesis or aspartic acid biosynthesis, respectively. Being able to predict how much oxalo acetate goes into which pathway requires knowledge of the concentration of oxalo acetate as well as the concentration and kinetics of each of these enzymes. This aim of predicting the behaviour of metabolic pathways reaches its most complex expression in the synthesis of huge amounts of kinetic and gene expression data into mathematical models of entire organisms. Alternatively, one useful simplification of the metabolic modeling problem is to ignore the underlying enzyme kinetics and only rely on information about the reaction network's stoichiometry, a technique called flux balance analysis (Almaas.E, *et al*, 2004).

α-Glucosidase Inhibition Enzyme Kinetics

The α-Glucosidase inhibition Enzyme Kinetics effect was measured using the method slightly modified from (Dahlqvist, 1964). The assay mixture consisted of 100 mM malate buffer (pH 6.0), various percentage sugar substrate solution (100µl) and 3 constant mM of inhibitor (Beta-Amyrin) different where added. It was pre-incubated for 5 min at 37 °C, and the reaction was initiated by adding the yeast α-glucosidase solution (50 µl) to it, followed by incubation for 10 min at 37 °C. The glucose released in the reaction mixture was determined with the kit GOD-POD (Glucoxidase-Peroxidase) by adding 10µl of sample to 1000 µl of GOD-POD reagent. Where the Glucose released by enzyme is been convert to Gluconic acid and H_2O_2 this will convert as Red quinone with 4 H_2O molecules by POD with help of 4-Amino anti-pyrine. The rate of carbohydrate decomposition was calculated as the percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The final product is perceived with help of UV spectrometer at 505nM. The same experiment was repeated with standard as Acarbose.

Michaelis-Menten and Lineweaver-Burk Model

The data obtained from α-Glucosidase inhibition Enzyme Kinetics was plotted in Michaelis–Menten plot in graph pad prism 6 and Lineweaver-Burk plot with Microsoft Excel 2007. This document reveals the V_{max}, K_m values and the type of inhibitors.

RESULTS

α-Glucosidase Inhibition Enzyme Kinetics (Fig. 3.1 & Fig 3.2) and (Table. 3.1 & 3.2) was revealed that Beta-Amyrin shows potent inhibition activity at V_{max}1.614±0.1825, K_m3.588±0.6432 at10.0mM but Standard Acarbose shows V_{max}1.603 ± 0.2073, K_m4.666 ± 0.8832 at 1.0mM itself. However Beta Amyrin shows very less inhibition compare other inhibitors. Interestingly all the Lineweaver-Burk plot (Fig 3.1 & Fig 3.2) spectacles that all the inhibitors are competitive to isolated α-Glucosidase because they have Y-intercept as uninhibited enzyme in different slopes and X-intercept between other data sets.

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion insulin action or both. Along with hyperglycemia and abnormalities in serum lipids, diabetes is associated with micro and macro vascular complications, which are the major causes of morbidity and death in diabetic subjects (Kumar and Murugesan, 2008). Management of the blood glucose level is a critical strategy in the control of diabetes and its complications. Diet rich in carbohydrate causes sharp rise in the blood glucose level as the complex carbohydrate in food is rapidly absorbed in the intestine aided by the α-glucosidase enzyme which break carbohydrate to simple absorbable sugars (monosaccharides) (Kwon, YI *et al*.,). Inhibitors of saccharide hydrolyzing enzymes (α-glucosidase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with Type II

diabetes mellitus (Gin.H & Rigalleau.V). These inhibitors delay carbohydrate digestion time causing reduction in the rate of glucose absorption and consequently reducing the post prandial plasma glucose rise (Bhandari M.R *et al*).

Synthetic hypoglycemic drugs like acarbose, miglitol and voglibose are used in conjunction with other anti-diabetic drugs, but these inhibitors have been found to possess gastrointestinal side effects like abdominal discomfort, flatulence and diarrhoea. As a result of this, there is growing interest in discovering new and effective α-amylase and α-glucosidase inhibitors from plants with minimal or no side effects (Shai L.J *et al*). The rate of carbohydrate decomposition was calculated as the percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The final product is perceived with help of UV spectrometer at 505nM and the Acarbose as standard. In the present study, Bioactive compound Beta Amyrin shows the potent inhibition at V_{max} 1.614 ± 0.1825, Km3.588 ± 0.6432 at concentration of 10.0 mM**.** However Beta Amyrin shows very less inhibition compare other inhibitors. In order for hypoglycemic agents to elicit their pharmacological effects and prevent the side effects experienced with the use of synthetic drugs, medicinal plants should be mild inhibitors of α-amylase (Kwon Y.I *et al*). The curve generated from the Lineweaver-Burke plot of this inhibition shows a near competitive mode of inhibition of the α-glucosidase. This implies the active components of the extract compete with the substrate for binding to the active site of the enzyme, thereby preventing or slowing down the breakdown of oligosaccharides to disaccharides (Shai L.J *et al*).

CONCLUSION

The present study we can conclude that plant medicines are showing a better inhibitory activity. Natural medicines have lesser or no side effects when compared with chemical inhibitors. The present study results reveals that the isolated bioactive compound Beta Amyrin of *Memecylon umbellatum* is showing better anti-diabetic activity. The results indicate that Beta Amyrin showed the maximum α-glucosidase inhibitory activity. Isolation of Bioactive compounds from medicinal plants is easier to produce and less expensive, Triterpenoides have a largest group of natural plant products. Bioactive compound Beta Amyrin which is isolated from *Memecylon umbellatum* used as α-glucosidase inhibition activity. The leaves and roots of the plant *Memecylon umbellatum* were screened for hypoglycemic activity. Oral administration of the alcoholic extract of leaves of Memecylon umbellatum showed a significant lowering of serum glucose levels in normal and alloxan induced diabetic mice (Amalraj.T and Ignacimuthu.S, 1998). α-Glucosidase inhibition Enzyme Kinetics was revealed that Beta-Amyrin shows potent inhibition activity. The Results of this study provide scientific support for the use of Memecylon umbellatum leaves in traditional medicine for the treatment of diabetes. This study should help to explain the pharmacological mechanism of action and also help in the development of novel medicinal preparations or functional foods for the treatment of diabetes and related symptoms.

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