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# EVALUATION OF SELECTED MEDICINAL HERBS FOR ANTIDIABETIC ACTIVITY VIA ALPHA-GLUCOSIDASE INHIBITION

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## ABSTRACT

Literature and native therapies have cited bitter melon, dandelion, blueberry, and roselle, as hypoglycemic agents, however, the exact mechanisms of action are unknown. It was hypothesized that, these agents could induce hypoglycemia, through the mechanism of α-glucosidase inhibition. The aim of the present study was, to examine inhibition of alpha-glucosidase as one of the possible mechanisms of action, of bitter melon (*Mormodicacharantia*), dandelion (*Taraxacumofficinale*), blueberry (*Vacciniumcorybosum*), and roselle (Hibiscus sabdariffa). Each of these agents has been used in the treatment of diabetes in, different parts of the world. The study was done *in vitro,* using α-glucosidase, obtained from Bacillus. The inhibitory effect of different concentrations of alcoholic extracts of the plants, on α-glucosidase was studied. The extracts of the plant showed inhibitory activities, against α-glucosidase, with IC50 values in a dose dependent manner. The result demonstrated that, bitter melon, roselle, dandelion, and blueberry share similar mechanism of action with Acarbose, which is being used as an antidiabetic agent.

**KEYWORDS:** Diabetes, Alpha-Glucosidase, Medicinal Plants, Mechanism of Action

## INTRODUCTION

Type 2 diabetes (T2D), formerly known as non-insulin dependent diabetes mellitus or adult-onset diabetes mellitus, is a chronic disorder of glucose equilibrium, which results from the body's inability to make use of available insulin, along with relative insulin deficiency. T2D constitutes approximately 90% of all diabetes cases, around the world; type 1 diabetes (T1D) and gestational diabetes make up the remaining 10% of the cases. Diabetes is one of the most common non-communicable diseases worldwide, and is an escalating public health problem, globally [1]. Diabetes has become a global epidemic, with significant impact on society and the economy. In 2013, about 382M people around the world were estimated to suffer from diabetes [2]. Major sources of the morbidity of diabetes are the chronic complications, that arise from prolonged hyperglycemia, including retinopathy, neuropathy, nephropathy, and cardiovascular disease. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities [3].

According to an estimate, one person is detected with diabetes every 5 seconds, somewhere in the world, while, someone dies of it every 10 seconds and a pathogenic relationship exists between type 2 diabetes and obesity [4]. In the United States of America, it is the 7$^{th}$ leading cause of death. Oral hypoglycemia has been the mainstay of treatment. Over the past seventeen years, many new therapeutic interventions have been introduced, including bariatric surgery, dipeptidyl peptidase-4 inhibitor, glucagone-like peptide-1 analogs, and cannabinoid receptor-1 antagonists [5]. Despite, the

progress made with synthetic drugs available, the search continues for newer drugs because, the existing ones have limitations. They produce many side effects and may precipitate noncompliance, by the patients. Plants are acceptable because, they may cause less side effects, may be cheaper, and may be more easily accessible. The World Health Organization (WHO), has approved the use of medicinal herbs for remedies. Though medicinal plants have been used historically, throughout the world, few of them have been validated scientifically. Acarbose, miglitol and viglibose have been introduced as alpha-amylase and alpha- glucosidase inhibitors. The inhibition of intestinal α-glucosidases, delays the digestion of and absorption of carbohydrates, and consequently suppresses postprandial hyperglycemia [6]. Alpha-glucosidase inhibitors also reduce triglycerides [7]. Because of these benefits, there has been extensive research for α-glucosidase inhibitors, from plant sources. Sources from plants, foodstuff, and bacteria have been studied [8]. In the search for potent α-glucosidase inhibitor, we focused on those medicinal plants, that are used traditionally for treating diabetes. These include Blueberry (*Vacciniumcorybosum*), Dandelion (*Taraxacumofficinale*), and bitter melon. Some studies reported efficacies of these plants, in the treatment of dysentery; gastrointestinal disorders, malaria, cancer, microbial and parasitic diseases [9, 10]. *Momordicachanrantia,* or bitter melon, is a plant indigenous to tropical areas. The uses of this plant include treatment of inflammation, hyperglycemia, hyperlipidemia, neoplasia, and even viral infections. It has been traditionally used for hypertension, and inflammation, among many other conditions [11]. Dandelion [12, 13, 14], grows in the Northern hemisphere, temperate zones. It is used for gastrointestinal ailments (dyspepsia and appetite loss). It is also used as a diuretic, and it is known to reduce hyperglycemia. Blueberry, (*Vacciniumcorybosum*), is usually from the east and north-central North America, it has been reported to possess antidiabetic activities [15]. In this study, we tested the efficacy of some of the medicinal herbs, used traditionally for diabetic treatment, for their ability to inhibit alpha-glucosidase, using Acarbose as the positive control.

## MATERIALS & METHODS

100 g of dry roselle calyces was homogenized with ethanol (400 ml), using a blender (food processor). The resulting mixture was stored for 72 hours at 4 °C, for extraction followed by vacuum filtration. The filtrate was evaporated to dryness, using an evaporator. In a similar way, 100g of each of the other plants, bitter melon (fruit), dandelion (leaves), and blueberry (fruit), used in this experiment was homogenized with ethanol, using a blender (food processor). The resulting mixtures were left to soak at 4 °C for 72 hours, followed by vacuum filtration. The filtrate was evaporated using a rotavapor.

### Materials

0.2M phosphate buffer, pH 6.8 purchased was from Alfa Aesar, 4-Nitrophenyl α-D-glucopyranoside, Acarbose, Albumin from bovine serum, and α-glucosidase from bacillus stearothermophilus, were purchased from Sigma-Aldrich. Bitter melon (fruit), Blueberry (fruit), Roselle calyces and dandelion leaves were obtained locally. They were identified by Xavier University, of New Orleans Botanist.

### Alpha-Glucosidase Inhibitory Assay

This assay was performed according to Ramirez [16], with some modifications. The *in vitro* assay was carried out in a 96-well microplate. The reaction mixture consisted of 2.5 µL of various dilutions of the extract, 122.5µl of 0.2M phosphate buffer and 62.5 µL of 5mM para-nitrophenyl-α-D-glucopyranoside. The mixture was pre-incubated at $37^0$C for

5 min, followed by the addition of 62.5 µL of α-glucosidase solution. The mixture was then incubated, for an additional 10 minutes and analyzed with a Thermo Scientific® Multiskan Spectrophometer, at an absorbance of 400nm. Acarbose was used as a positive control of α-glucosidase inhibitor, and was analyzed similarly. The experiments were done in triplicate and repeated over five times.

$$\% \text{ Inhibition} = \frac{A_{410} \text{ control} - A_{410} \text{ test}}{A_{410} \text{ control}} * 100$$

The $IC_{50}$ values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined, by performing the assay as above, with varying concentration of the plant extracts ranging 20 to 100 µg. The $IC_{50}$ values were determined from plots of percent inhibition vs log inhibitor concentration, and calculated by non-linear regression analysis from the mean inhibitory values.

## RESULTS

The results of this study are shown in figures 1-4. All the extracts inhibited the alpha-glucosidase, in a dose-dependent fashion. The $IC_{50}$values were as follows: Acarbose: 0.53E-5±3.59 mg/ml, Roselle: 0.917±0.26 mg/ml, Bitter melon: 0.31±0.06 mg/ml, and Blueberry 0.9599±0.178 mg/ml. The result shows that, each of the agents inhibited the alpha-glucosidase. The inhibition was dose dependent.

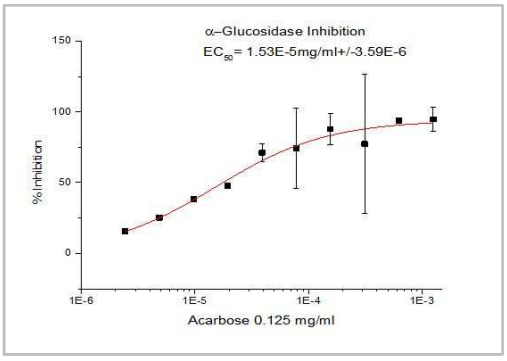


**Figure 1: Effect of Inhibition of Alpha-Glucosidase by Acarbose The Results are Expressed in Mean± Standard Deviation**

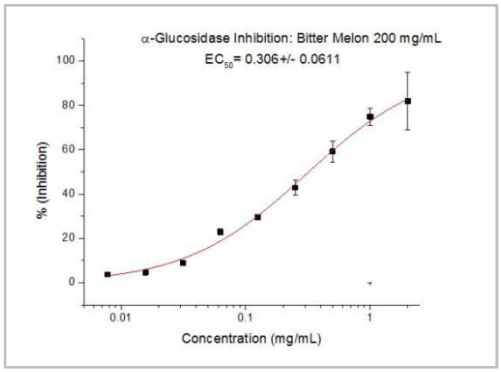


**Figure 2: Effect of Inhibition of Alpha-Glucosidase by Bitter Melon The Results are Expressed in Mean± Standard Deviation**

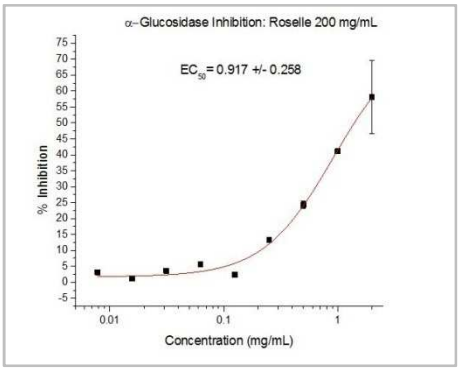


**Figure 3: Effect of Inhibition of Alpha-Glucosidase by Roselle The Results are Expressed in Mean± Standard Deviation**

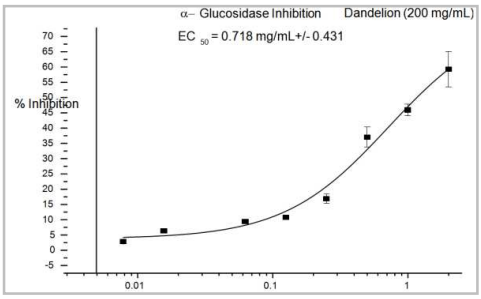


**Figure 4: Effect of Inhibition of Alpha-Glucosidase by Dandelion The Results are Expressed in Mean± Standard Deviation**

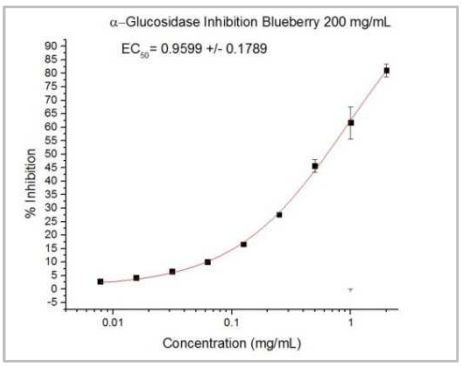


**Figure 5: Effect of Inhibition of Alpha-Glucosidase by Blueberry The Results are Expressed in Mean± Standard Deviation**

## DISCUSSIONS

The results of this study showed that, the alcoholic extracts of the plants used, blueberry fruits, bitter melon fruits, dandelion leaves and, roselle leaves, each inhibited alpha-glucosidase in a dose dependent manner. Acarbose is used in its purified form, whereas, the other extracts were used in their crude form. These compounds seem to offer some promise, as candidates for further investigation. It is hopeful that, when the active ingredients are isolated, they may prove to be inhibitory than the crude alcoholic extracts, used in this project. These agents are used in traditional medicine, for the treatment of diabetes, but their mechanisms of action are lacking. This study elucidates one of the mechanisms by which, the plants produce their hyperglycemia reducing effect. Diabetes is one of the most common non-communicable diseases worldwide, and is an escalating public health problem, globally. Since, α-glucosidase enzyme functions by breaking down complex sugar and carbohydrate from food into simple sugar, leading to increase in blood glucose postprandial surge in

type- 2 diabetes, inhibiting its activities will be of therapeutic benefit, in the treatment of type- 2 diabetes. This is in agreement with the work of scientists, researching in the area of drug discovery in diabetes [17, 18, 19]. The rate of new cases of diagnosed diabetes, in the United States has begun to fall, but the numbers are still very high. More than 29 million Americans (30.3 million) are living with diabetes, and 86 million are living with prediabetes [20]. Diabetes has become a global epidemic. There is a dire need to diagnose and treat the disease in time, in order to prevent complications. Though, advances have been made in this area, search for newer drugs continues, due to limitations posed by an adverse drug reaction. Hence, the search for more effective and safer hypoglycemic agents is still an important field of investigation. By showing an inhibition of α-glucosidase, these extracts may offer the potential to reduce blood glucose level, as required in type 2 diabetes treatments. This makes each plant a candidate, for more in vitro and in vivo studies, to explore their efficacy and safety profiles, for the treatment of diabetes.

## CONCLUSIONS

The mechanism of action of plant extracts, Blueberry (*Vacciniumcorybosum*), Dandelion (*Taraxacumofficinale*), and Bitter melon (*Taraxacumofficinale*) have been investigated. The alcoholic extracts, inhibited alpha-glucosidase *in vitro* in a dose dependent fashion. Further, studies will include isolating the active materials, by fractionation and testing the purified extracts, both *in vitro* and in *vivo* studies. Intensive research in drug discovery will help to reverse the global diabetic epidemic

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