In Vitro investigating the antidiabetic activity of *Rumex abyssinica, Hibiscus sabdariffa, and Cinnamomum zeylanicum*

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ABSTRACT

Background: Diabetes is a metabolic disorder symptomized by high blood glucose level. The treatment is associated with adverse side-effects regarding health and economic burdens of the disease. The difficulty in insulin injection, the non-compliance, prolonged treatment, makes to the diabetic patients to have a poor controlled blood glucose level particularly in resource limited settings. As alternative, people are using medicinal plants as they are cheap, readily available, and lesser side effects. Even though the need for herbal medicine are culturally and socially accepted but the toxicity, active principles, and mechanism of action are generally not known well so this study aims to investigate in vitro potential effects of *Rumex abyssinica, Hibiscus sabdariffa, and Cinnamomum zeylanicum* in glucose regulation.

Methodology: Different parts from the 3 plants were subjected to hot Soxhlet extraction using solvents with different polarity. The in vitro anti-diabetic activity of the extracts was investigated by measuring their effect on assays of α-amylase inhibition and glucose transport across yeast cells.

Result: The result of α-amylase inhibition reveled highest, significant (p<0.001) from aqueous extracts (0.5 mg/ml) of *Hibiscus sabdariffa* and *Rumex abyssinica* by 85.11%, and 77.91% respectively. Though chloroform extract of *Cinnamomum zeylanicum* was not significant exhibited 92.49% α-amylase inhibition at 0.5 mg/ml. In the glucose transport assay, the aqueous extract (0.5 mg/ml) of *Cinnamomum zeylanicum, Hibiscus sabdariffa*, and *Rumex abyssinica* increased glucose transport across yeast cells by 52.44%, 52.16%, and 50.57% respectively, at 25mM glucose concentration.

Conclusion: All the three selected medicinal plants were found to have an effective inhibition of α-amylase and glucose absorption by the aqueous extract. This shows that the plants extract contains an active water-soluble ingredients which may have anti-diabetic activity in vivo when taken in the form of tea. Therefore, we kindly recommend that, a further invivo, toxicological, phytochemical, and molecular analysis of these plants to be conducted in case control studies as it has been used as tea in the community.

KEYWORDS: In vitro, anti diabetic activity, inhibition, yeast uptake, medicinal plants

INTRODUCTION

Diabetes is a metabolic syndrome defined as a hyperglycemia caused by inadequate insulin production, insulin action, or both. Diabetes' persistent hyperglycemia is linked to long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, peripheral nerves, heart, and blood vessels [1]. Diabetes mellitus is classified into four types: type 1, type 2, gestational diabetes mellitus, and other particular types [2]. Diabetes mellitus is one of the world's most alarming diseases. Diabetes caused an estimated 2 million deaths in 2019, and it is a major cause of blindness, renal failure, heart attacks, stroke, and lower limb amputation [3]. Diabetes affected around 537 million individuals (20-79 years) in 2020, and this figure is expected to climb to 783 million by 2045. Furthermore, according to the International Diabetes Federation (IDF), 24 million individuals in Africa reported with diabetes in 2021, with a projected increase to 55 million by 2045 [4]. Similarly, the estimated number of adults (20-79 years old) living with impaired fasting glucose (IFG) in Eritrea is anticipated to rise from 107,300 in 2021 to 249,600 in 2045 [5]. The anti-hyperglycemic drugs used sofar have many modes of action that primarily aim to keep blood glucose concentrations closer to normal. Although these drugs alleviate symptoms and reduce diabetes-related mortality, they have a negative impact...
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on life quality [6]. In the process, research on medicinal plants as therapeutic aid is concentrated on plant and herbal drugs [7] [8], which have a history that predates modern medicine [9]. The plant based herbal medicine are thought to be safer than pharmaceutical medications [10]. According to ethnobotanical studies from around the world, there are over 1200 plants with anti-hyperglycemic potential [11]. In addition Alkaloids, glycosides, flavonoids, saponins, and other active components have been documented to have therapeutic properties [12]. The Eritrean traditional medicinal plants *Hibiscus sabdariffa*, *Cinnamomum zeylanicum*, and *Rumex Abyssinia* were listed for this investigation because of their historically stated anti-diabetic properties [13]. However, there is no scientific evidence supporting the claim in Eritrea, thus the study intends to evaluate the probable anti-diabetic potential of plant extracts using two in vitro models. Carbohydrate-digesting enzyme (α-amylase) inhibitory activity and yeast glucose absorption are the two in vitro methods used.

**MATERIALS AND METHODS**

Reagents and Chemicals

Chloroform, acetone, and, DMSO (purchased from BDH) alpha amylose (purchased from LD Carlson company, distributed by LD Carlson co, Kent, OH 44240), 3,5- dinitro salicylic acid (DNSA), sodium phosphate buffer, K+Na+ tartrate, NaOH (purchased from BDH), starch and glucose (purchased from fisher scientific).

Collection and Preparation of Plant Extracts.

The selected plants were purchased from the spices market in Asmara, Eritrea and authenticated by a botanist from the College of Science, Department of Biology, Mai-nefhi, Eritrea. The different plant parts: flowers, bark, and roots of *Hibiscus sabdariffa*, *Cinnamomum zeylanicum*, and *Rumex abyssinica*, respectively, were washed thoroughly and dried in a shaded area at room temperature (20-22°C) for seven days. The dried plant parts were then ground to powder-form using a grinding machine.

The active ingredients of the plants were extracted by continuous hot Soxhlet extraction using three different solvents with increasing polarity chloroform, acetone, or distilled water. The solvents were then evaporated, by a rotary evaporator, under reduced pressure and controlled temperature (20-22°C). These plant extracts were dried and stored in a clean petri dish and kept at 4-7°C for further use in the in vitro anti-diabetic assays.

Anti-diabetic Activity

The extracts of the flowers, bark, and roots of *Hibiscus sabdariffa*, *Cinnamomum zeylanicum*, and *Rumex abyssinica*, respectively, were tested for their inhibitory effect against the carbohydrate digestive enzyme (α-amylase) and their role in glucose uptake in yeast cells.

Inhibition of α-Amylase Enzyme.

This assay was carried out following the procedures by Yosief et al. [14]. Different concentration plant extracts (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml) were prepared. The plant extract (250 ul) were then added to 0.02M sodium phosphate buffer (pH 6.9) containing 0.15 mg.ml α-amylase. The solution was pre-incubated at 25°C for 10 min; 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added and the mixture was further incubated at 25°C for 10 min before the reaction was terminated by adding 2 ml, dinitro salicylic acid (DNSA) reagent. The test tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 ml distilled water and the absorbance was measured at 525 Cat 540 nm using a spectrophotometer. A control was prepared using the same procedure replacing the plant extract with distilled water. The α-amylase activity was calculated using the following formula: (1)

% Inhibition= Gc – Gi / Gi x100

Where Gi (control) is the glucose concentration of the control reaction and Gc (sample) is the glucose concentration of the test sample.

Glucose Uptake in Yeast Cells

Commercial baker’s yeast was washed three times with distilled water, and a 10% (v/v) suspension in distilled water was prepared. Different concentrations of plant extract were added into 1ml glucose solution and incubated at 37°C for 10 min. After 10 min of incubation yeast suspension was added and mixed by vortex to initiate the reaction. The reaction mixture was further incubated for 60 minutes at 37°C. Then, the tubes were centrifuged, and glucose uptake was immediately estimated in the supernatant using a spectrophotometer at 540 nm. The percentage increase in glucose uptake by yeast cells was calculated using the formula (1) [15].

Data Analysis
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The entire analysis was undertaken in triplicate. Quantitative values were presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to evaluate the statistical differences followed by Tukey HSD post hoc test.

**RESULT**

**α-amylase inhibition assay**

The in vitro α-amylase inhibitory activities of *Rumex abyssinica*, *Hibiscus sabdariffa* and *Cinnamomum zeylanicum*, extracted with three solvents were assayed as seen in Figure 1. The distilled water extract of the plant *Rumex abyssinica* exhibited significant inhibition in vitro α-amylase activity at all concentrations (p<0.001). The increase in inhibition by 77.91% in α-amylase activity was recorded highest at 0.5 mg/ml. Acetone extract of *Rumex abyssinica* (0.3 to 0.5mg/ml) showed significant (p<0.001) inhibition in α-amylase activity (by 45.49% to 64.33%). While the chloroform extract of the plant *Rumex abyssinica* inhibited in vitro α-amylase activity in a dose-dependent manner at concentrations ranging from 0.1 to 0.5mg/ml (2.96% to 71.46%). However, significant inhibition was recorded only at 0.5 mg/ml (p<0.001).

The aqueous extract of *Hibiscus sabdariffa* showed significant dose-dependent inhibition in vitro α-amylase activity (p<0.001) at all concentrations. While the acetone extract exhibited a linear dose response in vitro α-amylase inhibition ranging from 2.2% to 61.24% that was significant at concentrations higher than 0.3 mg/ml(p<0.001). The chloroform extract also showed dose dependent inhibition at the concentration range from 0.1 mg/ml to 0.5 mg/ml (3.41% to 58.83%). However, the increase in vitro α-amylase inhibition by chloroform extracts were significant at the concentrations 0.3mg/ml (p<0.01), 0.4 mg/ml and 0.5 mg/ml (p<0.001), Figure 2.

The distilled water extract showed significant (p<0.001) dose dependent inhibition in vitro α-amylase activity at concentrations higher than 0.3 mg/ml. The acetone extract of *Cinnamomum zeylanicum* exhibited inhibition ranging from 1.9% to 47.75%. Significant (p<0.05) inhibition was observed at the highest concentration 0.5 mg/ml acetone extract. The chloroform extract of the plant *Cinnamomum zeylanicum* showed linear increase in vitro α-amylase inhibition though the difference was not significant, Figure 3.

**Glucose Uptake in Yeast Cells**

Different concentrations of the selected plant extracts (0.1mg/ml, 0.2mg/ml, 0.3mg/ml, 0.4mg/ml, and 0.5mg/ml) were used to investigate their effect on glucose uptake in yeast cells at 5-, 10- and 25-mM glucose. Increase in glucose uptake in yeast cells was observed at glucose concentrations higher than 10 mM.

At 10mM of glucose concentration, *Rumex abyssinica* extract (0.5mg/ml) promoted increase in glucose uptake, (41.1 %, distilled water, 33.8 % chloroform and 34.1% acetone). At 25mM of glucose concentration, *Rumex abyssinica* extracts (0.1mg/ml to 0.5mg/ml) by the different solvents exhibited a dose dependent increase in glucose uptake. The highest increase in uptake at 25 mM of glucose concentration was recorded for the 0.5mg/ml aqueous (50.57%), followed by 43.02% for chloroform and 40.13% for acetone extract Figure 4.

At 10 mM glucose, the distilled water, chloroform and acetone, extracts of *Hibiscus Sabdariffa* (0.5mg/ml) promoted increase in uptake of glucose across the plasma membrane of yeast cells. The highest increase by 36.4%, 34.38%, 31.12% was recorded for 0.5 mg/ml in water, chloroform and acetone respectively. Similarly, at 25 mM glucose concentration, the highest activity was recorded at 0.5mg/ml extract concentration. The increase in uptake was by 52.16% in distilled water, 40.60% in chloroform and 43.37% in acetone extract, Figure 5.

At 10mM glucose the highest increase in uptake was reached at 0.5mg/ml concentration. The increase in uptake was by 43.05%, 23.2%, and 28.79%in destined water chloroform and acetone respectively. At 25mM of glucose concentration, the distilled water, chloroform and acetone plant extracts exhibited a dose dependent increase in glucose uptake from 0.1mg/ml to 0.5mg/ml concentration. The highest increase in uptake was recorded in 0.5mg/ml concentration by 52.44% distilled water, followed by 33.36% for chloroform and 40.01% for acetone extract Figure 6.

**DISCUSSION**

The enzyme alpha-amylase breaks down the alpha-bonds in polysaccharides like glycogen and starch to produce mono- and disaccharides like maltose and glucose. Alpha amylase inhibitors bind to the polysaccharide’s alpha-bond and unable it from dissolving into monosaccharides. It has been demonstrated that the rise in postprandial glucose levels is positively associated with the activity of alpha-amylase. Alpha-amylase activity is thus implicated in the control of postprandial hyperglycemia and perhaps in the management of type 2 diabetes [16].Plant extracts that impact glucose uptake across the plasma membrane are anticipated to affect glucose homeostasis given that diabetes is a condition with defective glucose uptake.
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Plants consumed in Eritrea that are well-recognized to have anti-diabetic effects include *Hibiscus sabdariffa*, *Cinnamomum zeylanicum*, and *Rumex abyssinica*. *Hibiscus sabdariffa* and *Cinnamomum zeylanicum* have been explored for their effects on α-amylase inhibition and glucose uptake in the past researches [17], [18], [19] [20]. However, *Rumex abyssinica* has not yet been examined for its effects on glucose regulation. Therefore, the purpose of the study is also to determine how *Rumex abyssinica* differs from *Hibiscus sabdariffa* and *Cinnamomum zeylanicum* in its ability to decrease α-amylase activity and glucose uptake across yeast cell membranes.

In this study all three plant extracts exhibited α-amylase inhibition. Highest inhibition was recorded in aqueous extract followed by acetone and chloroform extract. The α-amylase inhibition by *Rumex abyssinica* was similar to that of by *Hibiscus sabdariffa*, which was higher than the inhibition by *Cinnamomum zeylanicum*. Chloroform extract of *Cinnamomum zeylanicum* showed no significant inhibition, where as, the chloroform extract of *Hibiscus sabdariffa*, showed significant inhibition at concentrations higher than 0.3 mg/ml and *Rumex abyssinica* at 0.5 mg/ml.

Our findings on the effects of *Hibiscus sabdariffa* on α-amylase inhibition are consistent with previous research conducted by [17]. that suggested the plant's aqueous extract is a powerful inhibitor of pancreatic α-amylase [17]. Additionally, the pressurized water extraction of *Cinnamomum zeylanicum* resulted in inhibitory activity for α-glucosidase and α-amylase (42.8 mg mL-1 and 78.7 mg mL-1, respectively) (P 0.05) [18].

CONCLUSION

Based on the above findings, the more effective inhibition of α-amylase and glucose absorption by the aqueous extract show that the plant extract’s active ingredients are water-soluble. Hence, it’s possible that the extracts may have anti-diabetic activity in vivo when taken in the form of tea, the way they are usually consumed. Therefore, the observed results will provide a line for further research.

DECLARATIONS SECTION

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Authors’ contributions:

Helen Yemane, Nardos Tsegai, Sabela Ghirmay, and Diana Hadish conducted the study (contributed sample collection and preparation, sample analysis, and data interpretation). Helen Yemane and Nardos Tsegai drafted and conceived the manuscript. Helen Yemane was the one responsible for writing the manuscript and all contributed to revising and checking the manuscript. Dr. E. Tareke is responsible for the conception and design of the research, data interpretation, writing and revising, and finalizing of the manuscript. The authors are fully accountable for ensuring the integrity and accuracy of the work and the authors have read and approved the final manuscript.

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