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Hypoglycemıc Effectıveness of the Antıdıabetıc Propertıes of *RUMEX ACETOSA* and *SYZYGİUM CUMİNİ* Extracts on Normal and Alloxan Monohydrate-Induced Dıabetıc Rabbıts

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Prof. Raymond Jagessar obtained his BSc (Distinction) in Chemistry/Biology from the University of Guyana (1992) and his PhD from the UK (1995). He held three Post-Doctoral Research Fellowships (PDF) at the University of South Carolina, (USA), Wichita State University, Kansas (USA) and the University of the West Indies during the period, 1996-1999. He is also accredited with a distinction DiPEd (higher education) at UG in 2022. He has several international awards, amongst them are Chartered Chemist, CChem and Fellow of the Royal Society of Chemistry, FRSC, UK, member of the American Chemical Society, Research Grants locally and internationally etc. He is also a Fellow of the Caribbean Academy of Sciences and one of the awardees of the Guyana Innovation Prize, 2021. His research interests are broad, covering the spectrum of Pure and Applied Chemistry, Chemical Biology and Pharmaceutical Chemistry. He has published over one hundred (100) research articles, five book chapters, one book, three e-books and presented at over (100) international conferences, locally and internationally. He has given keynote presentations at several conferences. He was President of the Caribbean Academy of Sciences during the period, 2021-2023 and is currently Professor in Chemistry at the University of Guyana (South America) and Foreign Secretary of the Caribbean Academy of Sciences, CAS.

Achievements/ Award:

- **2022-2023:** One of The University of Guyana Institute of Research, Innovation & Entrepreneurship, UGIRIE Innovation Prize awardees
- **2022**: Dean's Honour Roll, Faculty of Education and Humanities, University of Guyana
- **2021**: One of five, awarded the Guyana Innovation Prize
- **2020-Current**: Fellow of the Caribbean Academy of Sciences
- **2020**: Long Service Award (20 years), Vice Chancellery, University of Guyana
- **2020**: One of the applicants selected to compete for the Guyana Innovation Prize
- **2019**: Pharmaceutical Nanotechnology and Nanomedicine, Certificate of Recognition of outstanding excellence to the Scientific Community, March 20-21, New York, USA
- **2019**: Excellent contribution to teaching and research in the Department of Pharmacy, bymUniversity of Guyana Students Pharmacy Association.
- **2016**: Promotion to the status of Professor, University of Guyana
- **2015**-2016: Promotion to Reader (Associate Professor) at the University of Guyana
- **2014**: Award of University of Guyana Science and Technology Support Project Research Grant from the World Bank.
- **2012**: Award of Fellow of the Royal Society of Chemistry, FRSC, UK
- **2009**: Research Grant from the Research and Publication Committee of the University of Guyana.
- **2009**: Indefinite Tenure, University of Guyana
- **2007-2008:** Award of TWAS (The World
- Academy of Sciences) Fellowships,to continue research in Natural Products Chemistry during sabbatical in the Department of Chemistry, University of West Indies, St.Augustine Campus Trinidad and Tobago
- **2007-2009**: Research grant from the Royal Society of Chemistry, United Kingdom. This award was won three consecutive years.
- **2007**: Award of Chartered Chemist, CChem from the Royal Society of Chemistry, England.
- **2006**: Research Grant from the Research and Publication committee of the University of Guyana.
- **2005**: Promotion to Senior Lecturer Status, University of Guyana.
- **2004**: Award of Cariscience Grant to conduct research in Natural Products Chemistry, University of the West Indies, St. Augustine campus.
- **2004**: Visiting Post-Doctoral Research Scholar, Department of Chemistry, University of Minnesota, USA.
- **1999**: Post-doctoral research fellowship and Part-time Lectureship, University of the West Indies, Barbados: Duration:1 year
- **1998**: Post-doctoral research fellowship, University of South Carolina. Duration 1 year.
- **1995**: Post-doctoral research fellowship, Wichita State University, Kansas. Duration 2 years.
- **1995**: PhD/DPhil, University of Oxford Inorganic Chemistry Laboratory.
- **1992**: Oxford International PhD Full Scholarships from Oxford University: Duration: 3 years.
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Content:

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Abstract

Diabetes mellitus is a chronic non-communicable metabolic condition associated with disruption of glucose metabolism and is found to be prevalent among the Guyanese population. This research explores insulin secretion and the antidiabetic potential of the natural remedies, *Rumex acetosa* (Sorrel)/ *Syzygium cumini* (Jamun) aqueous leaf extract in lowering the concentration of blood glucose in Alloxan Monohydrate-induced diabetic rabbits. The 12 rabbits acquired were divided into four (4) equal groups in which each group were placed in a separate cage. However, groups B, C, and D were made diabetic by administering Alloxan Monohydrate at a dose of 150mg/kg solubilized in 2.5 - 4 ml distilled water, which was used in the experiment after 48 hours. The remaining group, A, was known as the control group. All the groups were fed a standard diet (feed pellets and water), with addition of Glibenclamide to group B, and a definite volume of the aqueous extract (leaves of sorrel/jamun) to group C and D. At the end of this experimental research, based on results obtained, the antidiabetic properties/hypoglycemic effectiveness of the aqueous plant extract was deduced as compared to synthetic antidiabetic medications.

Keywords: Phytoconstituents, *Rumex acetosa* (Sorrel), *Syzygium cumini* **(**Jamun), hypoglycemic, Alloxan Monohydrate, Glibenclamide, Type1 Diabetes Mellitus, Type 2 Diabetes Mellitus, Insulin, lipid, beta-cells.

Introduction

Diabetes Mellitus is a heterogenic metabolic disease characterized by a significantly high level of glucose in the blood (hyperglycemia) due to either impaired insulin secretion or dysfunctional insulin. It's classified into Type 1, Type 2, Gestational Diabetes, Neonatal Diabetes, Maturity-onset Diabetes of the Young and Steroid-induced Diabetes. Type 1 Diabetes is more commonly found in the young population while Type 2 Diabetes is more commonly found in the elderly. In type I diabetes, the body completely stops making insulin. Type 1 diabetes requires daily insulin injections (or the use of an insulin pump) to stay alive. This type of diabetes most commonly affects children and young people, but it can strike anyone at any age. The cause of Type 1 diabetes is an auto immune system disease. The body attacks and destroys insulin-producing β-cells in the pancreas. Without insulin to allow glucose to enter the cells, glucose builds up in your bloodstream **[1, 2, 3, 4, 5]**.

In type 2 diabetes, adult-onset or non-insulin-dependent diabetes, the body produces insulin, but the cells do not respond to it as they should i.e. there is insulin resistance. The pancreas should produce more insulin in response to this insulin resistance, but this does not happen in type 2 diabetes. Hence, the glucose level rise in the blood stream. Because of these two issues, insulin resistance and difficulty producing more insulin, the insulin action is insufficient to transfer glucose from the bloodstream into the cells. Type 2 diabetes is more common in adults over the age of 40 who are overweight and have a family history of diabetes, while it is becoming increasingly common in younger people, including teenagers. Type 2 diabetes is a global epidemic.

What are the causes of diabetes? Obesity is the major driver of Diabetes around the world where studies have shown an increase from 1.3 billion obese persons in 2005 to 2 billion in the year 2030 since high obesity and low muscle mass leads to high insulin resistance[6].

Smokers have a 45% increased risk than non-smokers of developing Diabetes and smoking was also found to be a risk factor to obesity thus an increased risk for insulin resistance. In developing countries, 50 – 60% adult males were found to be smokers[6].

Diet plays a very important role in the development of this disease as carbohydrates and fats were found to be drivers of the disease, particularly, *trans*-fat. Also, beverages that are sugar sweetened increases the risk of developing Diabetes as persons with a high sugar-sweetened beverage intake was found to have a 26% greater risk of Diabetes. In most developing countries, due to changes in the social and economic status, there are resulting dietary changes that have a significant impact on weight gain. Other ways in which changes in diet has impacted the prevalence of Diabetes include: shifting from grains to polished rice and refined wheat, higher intake of meats, edible oils and added sugars and reduced intake of cereals **[6]**.

Symptoms of Diabetes include: frequent urination, dehydration, weight loss, hunger, blurry vision, numbness or tingling hands or feet, tiredness, heart disease, hypertension, stroke, kidney failure, lower limb amputations and being more to frequent infections **[7]**. It's characterized by elevated blood glucose level. Diabetes is said to impact all social, economic and ethnic backgrounds **[8]**. Hyperglycemia manifests in various forms with a varied presentation and results in carbohydrate, fat, and protein metabolic dysfunctions.

The prevalence of diabetes is typically higher among men than women but the Caribbean is exceptional in its unique over-representation of women versus men with diabetes. Access to cheap diabetes medication, such as insulin, is crucial for persons with the disease's survival. By 2025, a global agreement has been reached to halt the rise in diabetes and obesity. Diabetes affects around 422 million people globally, the majority of whom live in low- and middle-income countries, and diabetes is directly responsible for 1.5 million fatalities per year. Over the last few decades, both the number of cases and the prevalence of diabetes have significantly increased, (WHO, 2020)[9]. Nearly 26 million people in the United States are considered to have diabetes, yet only about 19 million have been diagnosed, leaving millions more untreated. If current trends continue, one in

every three adults in the United States will develop diabetes by 2050, according to the Centers for Disease Control and Prevention (CDC) **[7]**.

There are an estimated 463 million adults with diabetes worldwide and the International Diabetes Federation Atlas reported that the Caribbean region has the world's highest raw prevalence of diabetes at 13.3%. Treatment for Type 2 diabetes include medications (both for diabetes and for conditions that are risk factors for diabetes), insulin and lifestyle changes such as losing weight, making healthy food choices and being more physically active.

Diabetes also has a financial impact on the health-care system, since it accounts for one out of every ten dollars spent on medical care. Diabetes patients' medical costs are approximately twice as high as those of non-diabetic people. Furthermore, minority races and ethnicities are more likely to develop Type 2 diabetes, as indicated by Hispanics having a 66 percent higher risk and non-Hispanic blacks having a 77 percent higher risk than non-Hispanic whites **[6]**.

Around the world, Diabetes can be seen in most countries despite being low, middle or high-income countries and studies have shown the disease on a continuous rise despite efforts being made to keep it under control. There are some very common contributing factors that can be seen in every country and these include dietary changes, obesity, lack of exercise and smoking **[6]**.

Treatments for diabetes depend on the type of diabetes, how well controlled blood glucose levels are and other existing health conditions. For Type 1 diabetes, insulin must be taken every day because the pancreas no longer makes insulin **[1-5].**

Studies have proven that increased physical activity reduces Diabetes risk. Studies have also shown that watching television for two hours a day contributes to a 14% increase in diabetic risk, two hours of walking or standing contributes to 12% decreased risk and one hour of brisk walking contributes to a 34% decrease in Diabetic risk. Also, with the increased amount of vehicles being used today, there has been a 1.8kg weight increase thus eventually leading to obesity in persons who own a vehicle **[6]**.

Managing Diabetes occurs through education, carbohydrate and caloric restriction in the diet, exercise of more than 150 minutes every week and through close blood glucose monitoring. Treatment options include daily insulin administration, biguanides (Metformin), sulfonylureas, meglitinides, thiazolidinediones, etc. In obese patients, bariatric surgery may be a form of treatment **[10]**. If left untreated, Diabetes Mellitus can lead to many other complications such retinopathy, neuropathy, nephropathy, atherosclerotic cardiovascular disease, hypertension, dyslipidemia, stroke, myocardial infarction, blindness, renal disease, etc. These complications have been found to increase mortality risk.

Diabetes in the world increased significantly from 108 million in 1980 to 422 million in 2014 with the highest prevalence in low- and middle-income countries **[8]**. It was found that, in 2017, 6.28% of the world's population was affected by this disease, which amounts to 462 million persons **[12]**. According to the World Health Organization, Diabetes Mellitus was the main cause of 1.5 million deaths in the year 2019 with 48% of all deaths occurring before the age of 70. Approximately 9 million persons in the world suffer from Type 1 Diabetes while 95% of all diabetes cases in the world was that of Type 2 Diabetes. Women with Gestational Diabetes, along with their children, have an increased risk of developing Type 2 Diabetes later in their lives **[11]**.

There has been an extreme increase of diabetes cases in Guyana for the last 20 years, in both males and females, which makes it an everyday rising issue. According to the latest WHO data published in 2018, Diabetes Mellitus Deaths in Guyana reached 546 or 9.18% of total deaths. The age adjusted Death Rate is 101.17 per 100,000 of population ranks Guyana #8 in the world (Diabetes Mellitus in Guyana, n.d.). In Guyana, diabetes affected 9.1 percent of the population in 2016, with 10.9 percent of females and 7.2 percent of males suffering from the disease. There was also a 9% diabetes-related mortality rate in the country. Indo-Guyanese, Afro-Guyanese, and Amerindians were the ethnic groups most affected in Guyana, with rates of 43 percent, 30 percent, and 9 percent, respectively **[13].**

Diabetes has a financial impact on the health-care system, since it accounts for one out of every ten dollars spent on medical care. Diabetes patients' medical costs are approximately twice as high as those of non-diabetic people. Furthermore, minority races and ethnicities are more likely to develop Type 2 diabetes, as indicated by Hispanics having a 66 percent higher risk and non-Hispanic blacks having a 77 percent higher risk than non-Hispanic whites **[14]**.

It was discovered that overtime, diabetic medications have been causing life-threatening side effects by organ damage and some medications are becoming less effective and more expensive. Guyana is recorded as a third world country because of its economic background so this experiment was intended to help persons who are financially inclined by using herbal supplements with possible hypoglycemic properties such as Jamun (*Syzygium cumini*) leaves and Sorrel (*Rumex acetosa*) leaves as another way to tackle Diabetes.

Plant extracts are also safe to use. Medical plants, according to published studies, are more inexpensive and have less side effects than synthetic medications, and are more effective in the treatment of diabetes mellitus **[15]**.

Around the world, various plant extracts have been used since they are believed to have hypoglycemic properties. Some of these include *Brassica oleracea* (Wild Cabbage) and *Citrus limon* (Lemon) in Congo, *Pueraria lobate* (Kudzu) and

Rosa rugose (Japanese Rose) in China. Others include *Allium sativum* (Garlic) and *Olea europaea* (Olive) **[16]**.

Thus, the objectives of this research were to deduce whether *Rumex acetosa* (Sorrel) extract and *Syzygium cumini* (Jamun) can be used as a safe hypoglycemic agent in rabbits.

Some of the research questions and Null/Alternate hypotheses were:

1. Will *Rumex acetosa* (Sorrel)/ *Syzygium cumini* (Jamun) extracts have Antidiabetic activity?

a) **Null Hypothesis ($H_0$1):** *Rumex acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) leaf extract will not be effective in improving (lowering) blood glucose concentration in Alloxan Monohydrate-induced diabetic rabbits comparing with Glibenclamide.

b) (b)**Alternative Hypothesis (H_1):** *Rumex acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) leaf extract will have a positive outcome in improving (lowering) the concentration of blood glucose in Alloxan Monohydrate-induced diabetic rabbits comparing with Glibenclamide.

2. Will *Rumex acetosa* (Sorrel)/ *Syzygium cumini* (Jamun) extracts Antidiabetic activities be concentration dependent?

a) **Null Hypothesis (H_0):** The way in which the rabbits respond to *Rumex acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) extracts will not depend on the concentration given as compared with Glibenclamide.

b) **Alternative Hypothesis (H_1):** The way in which the rabbits respond to *Rumex acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) extracts will depend on the concentration given as compared with Glibenclamide.

3. Will *Rumex acetosa* (Sorrel)/ *Syzygium cumini* (Jamun) extracts be toxic to rabbits?

a) **Null Hypothesis (H_0):** *Rumex Acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) leaf extract will not be toxic to rabbits.

b) **Alternative Hypothesis (H_1):** *Rumex Acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) leaf extract will be toxic to rabbits.

4. Can *Rumex acetosa* (Sorrel)/ *Syzygium cumini* (Jamun) extracts be commercialized?

a) **Null Hypothesis (H_0):** The efficacy and potency of *Rumex acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) leaf extract will be ineffective to render it eligible to be commercialized for use.

b) **Alternative Hypothesis (H_1):** The efficacy and potency of *Rumex acetosa* (Sorrel)/ *Syzygium cumini* **(**Jamun) leaf extract will render it eligible to be commercialized for use.

Literature Reviews on *Syzygium cumini*

According to Raza *et al*., in 2017, the presence of non-nutritive secondary metabolites known as phytochemicals, is found in the seed and fruit of the Jamun plant which contain a variety of biologically active moieties which helps to reduce the risk of chronic disorders. The authors chose Secondary Data Analysis as their research approach since they relied on information from other authors and sources. The authors used statistical data/standard deviation to represent their conclusions (percentages). The ANOVA and the Duncan's Multiple Range test were applied. This article is incredibly valuable to humanity since jamun seed and fruit extracts were found to be helpful in managing blood glucose and insulin levels. Jamun is a natural herb with anti-diabetic properties **[17]**.

According to Chauhan & Intelli *in 2015*, Jamun fruit seeds and pulp have been shown to help diabetics lower their blood glucose levels. Due to its anthocyanin-rich, dark-purple flea- repellent pulp, jamun is most commonly used as an adjuvant therapy in type-2 diabetes. The research method used by the authors was decided to be Secondary Data Analysis- simply because the authors used information from other authors/sources. The research was carried out with the aim to find out the biochemical estimation of moisture, of crude fibre, iron and total protein of Jamun, which the authors were able to do **[18]**.

Antidiabetic activity of Jamun has been noted in Ayurvedic pharmacopoeia, which claims that the seed powder of Jamun is useful in managing high blood sugar levels," according to Jagetia in 2018. The research is relatively new as it dates back to 2018 and where originality is concerned, the authors used information from other sources/publishers which makes this article/research unoriginal. The research method used by the authors was decided to be Secondary Data Analysis- simply because the authors used information from other authors/sources. To represent their conclusions, the writer used representative tables and different samples to show the effectiveness of the Jamun plant for diabetes. This research is useful to humanity because "Jamun stimulates the activation of many enzymes such as catalase, glutathione peroxidase, glutathione-s-transferase, and enhance glutathione synthesis, as well as depletes lipid peroxidation, which may aid in lowering blood sugar cholesterol levels **[19].**

"The -amylase inhibitors of different portions of the Jamun plant provide an efficient antidiabetic method," according to Gajera *et al*. in 2017. The authors chose Secondary Data Analysis as their research approach since they relied on information from other authors and sources. To demonstrate the efficiency of the Jamun plant for diabetes, the writer employed statistical analysis/standard deviation, such as tables, detailed illustrations, and varied samples. "The data was analyzed using a two-factor full block randomized design

(2FCRD) (F1-Landraces; 2F-Fruit sections) as statistical methods for interpreting antidiabetic and antioxidant activity data," according to the press release. SPSS software was used to examine the data." Because the major goal of the study was to discover the varied compositions of each portion of the Jamun plant and how they separately work to lessen diabetes, this research is beneficial to humanity **[20].**

Syzygium cumini bark has long been used to cure diabetes in Ayurveda and Indian traditional medicine," according to Perera *et al.* in 2017. The objectives of this study was to find and validate the presence of anti-diabetic compounds in *Syzygium cumini* decoction, as well as to see if such compounds were available in a ready-to-drink produced from *S. cumini* decoction. The study is very new in terms of originality, the writers borrowed information from other sources/publishers, making this article/research unoriginal. The authors chose Secondary Data Analysis as their research approach since they relied on information from other authors and sources. To illustrate the usefulness of the Jamun bark for diabetes, the writer employed statistical analysis/standard deviation, such as tables, detailed illustrations, and diverse samples. Because the major goal of the research was to create a ready-to-drink concoction, it is beneficial to humanity **[21].**

Comparison of Journal Articles on *Syzygium cumini*/JAMUN

When compared, the five articles presented on *Syzygium cumini*/Jamun were all successful in proving that Jamun can be used to cure diabetes and has been doing so for centuries now. They all came to the same conclusion that almost every part of the Jamun plant can and has been used to treat chronic diseases such as Diabetes Mellitus. All five of the compared articles used Secondary Data Analysis to represent the findings in their research. However, they all used different test methods to arrive at their conclusions. Four of the articles presented all used statistical analysis/standard deviation to represent their data. The article written by Raza *et al*., in 2017, used the Anova Test along with the Duncan Multiple Range Test while the articles written by Jagetia, in 2018, Gajera *et al*., in 2017 and Perera *et al*., in 2017 used tables, graphs and various samples to represent their data findings. The other article written by Chauhan & Intelli in 2015 used the ASTA Method to demonstrate their data findings on the benefits of Jamun in diabetes **[18]**.

Literature reviews on *Rumex* Genus

An original research conducted by Aghajanyan *et al*, in 2018, was done to investigate antihyperglycemic and biochemical properties of *Rumex obtusifolius L*. in hyperglycemic rabbits. This was an experimental research which found that ethanol extract of *Rumex obtusifolius L*. seeds showed significant effect on hyperglycemia, with a reduction of 57.3% of fasting glucose levels while also improving glucose tolerance, and increasing liver glycogen content 1.5 fold. The extract also showed reductions in total cholesterol, low-density lipoprotein cholesterol levels and liver enzymes levels (alanine aminotransferase and aspartate aminotransferase) while there was an increase in high-density lipoprotein cholesterol levels. To represent their conclusions and findings, the writers used statistical analysis/standard deviation such as percentages of the different samples, tables and line graphs to show the effectiveness of the Biochemical Activity and Hypoglycemic Effects of *Rumex obtusifolius L*. Seeds **[22].**

Ahmad *et al* evaluated *Rumex hastatus* for its antidiabetic potential and nutritional purposes. It was an original experimental research which indicated that *Rumex hastatus* contains various compounds that are anti-diabetic namely butyl phthalate, phytol, ethylthreonine, dihydrobenzofuran, indoline, guanidine, nerolidol, myristic acid, palmitic acid, caryophyllene and anozol. Results also show that *Rumex hastatus* has an IC_{50} value of 42.09 µg/ml. Statistical methods such as Two-way ANOVA, Bonferroni Post Test, Standard Error Mean, GraphPad Prism and EL sheet were used to analyze information obtained indicating that the authors were successful in achieving their objectives of the research. This experiment can be very useful since the results can be used for future research and it also show that the plant species has a high anti-diabetic potential **[23].**

In 2020, Özenver *et al* investigated the antidiabetic profiles of the various extracts and phytochemicals of *Rumex acetosella L*. To represent their conclusions and findings, the writers used α- amylase and α-glucosidase assays by One-way ANOVA in addition to Tukey's Multiple Comparison test and Mean ± Standard Deviation to evaluate the Inhibitory potential on key enzymes relevant to type II diabetes mellitus and antioxidant properties of the various extracts and phytochemical constituents from *Rumex acetosella L*. Results indicated that ethanol and ethanol-water extracts inhibited α-glucosidase but showed little to no inhibition on α-amylase. This inhibitory action on α-glucosidase, along with the antioxidant potential of alcohol-including extracts indicate that *Rumex acetosella L.* can be used as an antidiabetic agent. These results can be very useful since its results can be used in future studies and also because the results indicate that *Rumex acetosella L.* extracts has a high potential as antihyperglycemic agents which can be utilized in the prevention and treatment of Diabetes Mellitus **[24].**

Khatri *et.al* investigated *in vitro* α-amylase inhibition activity and *in vivo* hypoglycemic and analgesic activity of ethanolic root extract of *Rumex nepalensis* and results obtained indicated an inhibition of α-amylase with IC_{50} value 912.22 µg/ml. However, this inhibition was dose dependent and showed significant reduction in blood glucose level in both normal and hyperglycemic rats. *Rumex nepalensis* also showed 68.72% analgesic activity. The objectives of this research were successfully achieved and results were analyzed using statistical methods such as Mean ± Standard Deviation along with Student's t-test in Microsoft Excel 2007 **[25].**

An original experimental study was conducted by Minh *et al*. This research was based on the isolation and purification of active components from the root of *R. crispus*, and the

evaluation of their anti-radical, anti-gout and anti-diabetic properties. The results of the study indicate that the compounds chrysophanol and physcion extracted showed significant inhibition of α-glucosidase with IC_{50} values of 20.1 and 18.9 µg/mL respectively. Chrysophanol and physcion also showed inhibition against the activity of xanthine oxidase at IC_{50} values of 36.4 and 45.0 µg/mL respectively. The authors successfully achieved the objectives of the research and analyzed the results using statistical methods such as One-way ANOVA and Tukey's post hoc test in Minitab Software. This experiment can prove to be very useful since results indicate that *Rumex crispus* root extracts can be used in the treatment of Diabetes Mellitus and Gout **[26].**

Comparison of Journal Articles on *Rumex* Genus

Several articles were researched which investigated hyperglycemic and biochemical properties of *Rumex* Genus in rabbits. In these original research, statistical methods were used. In the first article, the author used standard deviation such as percentages of the different samples, tables and line graphs, the second article utilized Mean ± Standard Deviation, whereas in the third article, One-way ANOVA, in addition to Tukey's Multiple Comparison test and Mean ± Standard Deviation, was used, in the fourth article One-way ANOVA and Tukey's post hoc test was used and lastly the fifth article two-way ANOVA were used to analyze the findings. Nevertheless, the authors were all successful in achieving their research objectives. Similar to, *Rumex* extracts has a high potential as antihyperglycemic agents, which can be utilized in the prevention and treatment of *Diabetes Mellitus*. These results can be very useful since its findings can be used in future studies.

Research Design

Part A

The research design used in Part A of this project, was experimental and correlational, since the aim of this research was to compare and analyze the hypogylcemic properties of two plant extracts (leaves of both *Rumex acetosa* and *Syzygium cumini*) with a synthetic drug, Glibenclamide.

Research Methodology

Fresh leaves were collected, washed, weighed, cut into pieces and blended in an electric blender with distilled water, avoiding spillage. After blending, the contents of the blender were filtered using Whatman Filter paper, with the resulting filtrate representing the plant extract filtrate. For Extract 1 (Sorrel), 97 grams of sorrel leaves were weighed and then placed into an electric blender. After placement of leaves, 473 grams of water was added to the blender. For Extract 2 (Jamun), 173 grams of Jamun leaves were weighed and then placed into an electric blender. After placement of leaves, 473 grams of water was added to the blender.

The plant extracts was stored in the refrigerator with a shelf life of three weeks after which fungus may develop. A total of twelve rabbits were used in the experiment, that is, three per cage resulting in four cages, since the experiment was done in triplicate. It was ensured that the cages were well cleaned and disinfected before conducting this experiment. All animals were marked. Prior to the start of the experiment, fasting blood glucose levels was taken in the morning using the glucometer by feeding the animals at 6pm the night before and removing the feed trough until the next morning after taking the blood glucose levels. All rabbits were divided into four groups: Group A (Control group), Group B, Group C and Group D, with Group B, Group C and Group D being rabbits made diabetic using Alloxan monohydrate at a dose of 150mg/kg solubilized in approximately 2.5 to 4 ml saline solution. Group A were normal rabbits not made diabetic. Diabetic groups (B, C and D) were used 24 hours after administration of Alloxan monohydrate/Saline solution. This was indicated by glucose level greater than 140 mg/dl using a glucometer. Group A represented non-diabetic (about 120 mg/dL) rabbits being fed nothing but normal feed and water, Group B represented rabbits that were diabetic (more than 140 mg/dL) and received normal feed and water along with doses of Glibenclamide, Group C represented rabbits that were diabetic (more than 140 mg/dL) and given normal feed and water along with doses of Aqueous Extract 1 (Sorrel) and Group D represented rabbits that were diabetic (more than 140 mg/dL) and given normal feed and water along with doses of Aqueous Extract 2 (Jamun). The aqueous extracts were administered at 6mL/kg per day. All groups were fed normal feed and water for the period of two weeks [27]. A common question that may arise is, why choose Glibenclamide instead of Metformin? Glibenclamide is a very potent anti-diabetic medication in the class of Sulfonylureas. Its high potency is due to the fact that it increases the secretion of insulin from the pancreatic beta-cells when it binds to receptors on these beta-cells of potassium ATP-dependent channels causing them to become depolarized[28]. Metformin, on the other hand, is in the class if biguanides which acts to reduce blood glucose levels through a reduction of glucose production in the liver, an increased sensitivity to insulin and by decreasing glucose absorption in the intestines[29]. Since Glibenclamide works directly on the beta-cells in the pancreas while Metformin works indirectly, it was the drug of choice in this experiment. The experiment proceeded for a period of two weeks, with blood samples being taken, with a glucometer, every two days resulting in samples taken four times every week thus having twelve readings in triplicates at the completion of the experiment in 21 days. The results obtained from the experiment were analyze whereby the Mean, standard deviation and Confidence Interval were expressed. The statistical method used to analyze the experimental result is the Analysis of Variance (ANOVA). All data analyses were done using Microsoft Excel 2016.

Research Population

The study population was strictly animal subjects that were bought from a Pet Shop located in Georgetown. The

study subject was twelve (12) rabbits varying in sexes of nine (9) males and three (3) females.

Results from Experiment

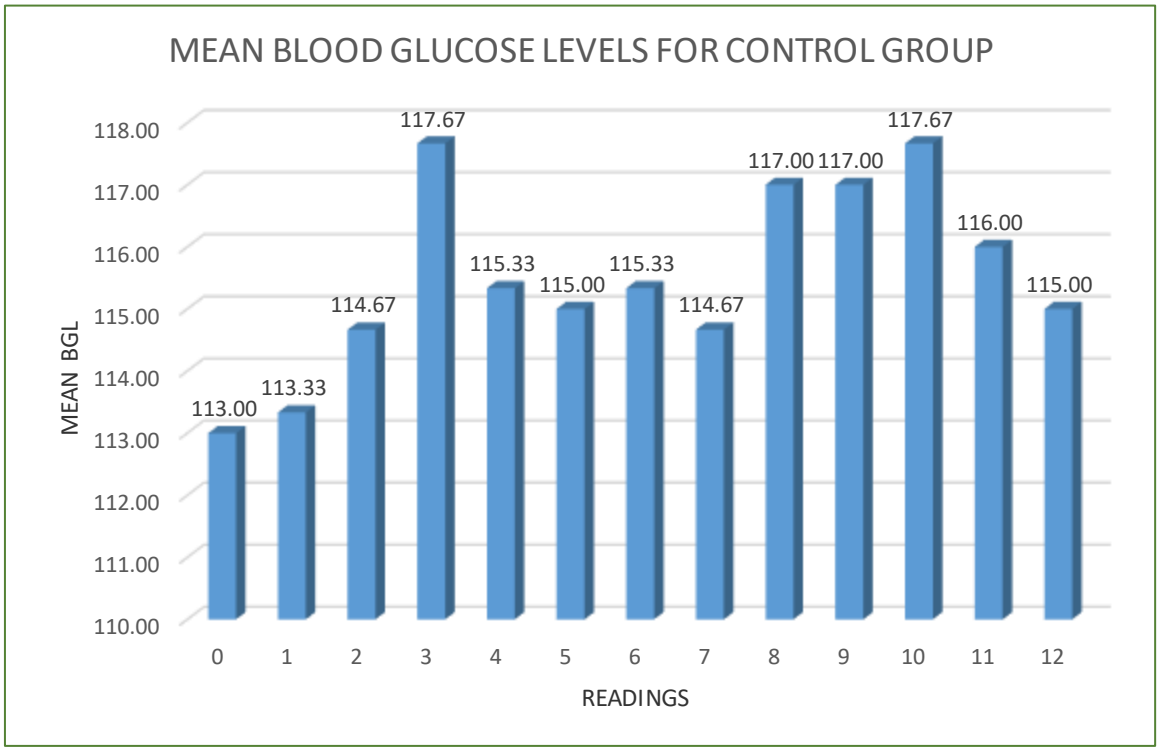
Table 1: Table showing the Blood Glucose Levels of Rabbits throughout experiment

	Group A (Control)				Group B (Glibenclamide)			
Readings	1	2	3	X	1	2	3	X
Initial	110	115	114	113 ± 2.65	114	128	150	130.67 ± 18.2
1st	112	115	113	113 ± 1.53	162	131	202	16.5 ± 35.6
2nd	114	113	117	114.67	162	131	129	140.67 ± 18.5
3rd	115	118	120	117.67 ± 2.52	148	134	146	142.67 ± 7.57
4th	115	116	115	115.33 ± 0.58	160	141	196	165.67 ± 27.93
5th	112	118	115	115 ± 3.0	177	136	148	153.67 ± 21.08
6th	119	114	113	115.33 ± 3.21	138	126	157	140.33 ± 15.63
7th	113	117	114	114.67 ± 2.08	159	116	148	141.0 ± 22.34
8th	117	118	116	117 ± 1.0	128	0	177	101.67 ± 91.39
9th	119	115	117	117 ± 2.0	117	0	135	84 ± 73.30
10th	115	118	120	117 ± 2.52	0	0	120	40 ± 69.28
11th	113	120	115	116± 3.60	0	0	0	0 ± 0
12th	119	115	111	115 ± 4	0	0	0	0 ± 0

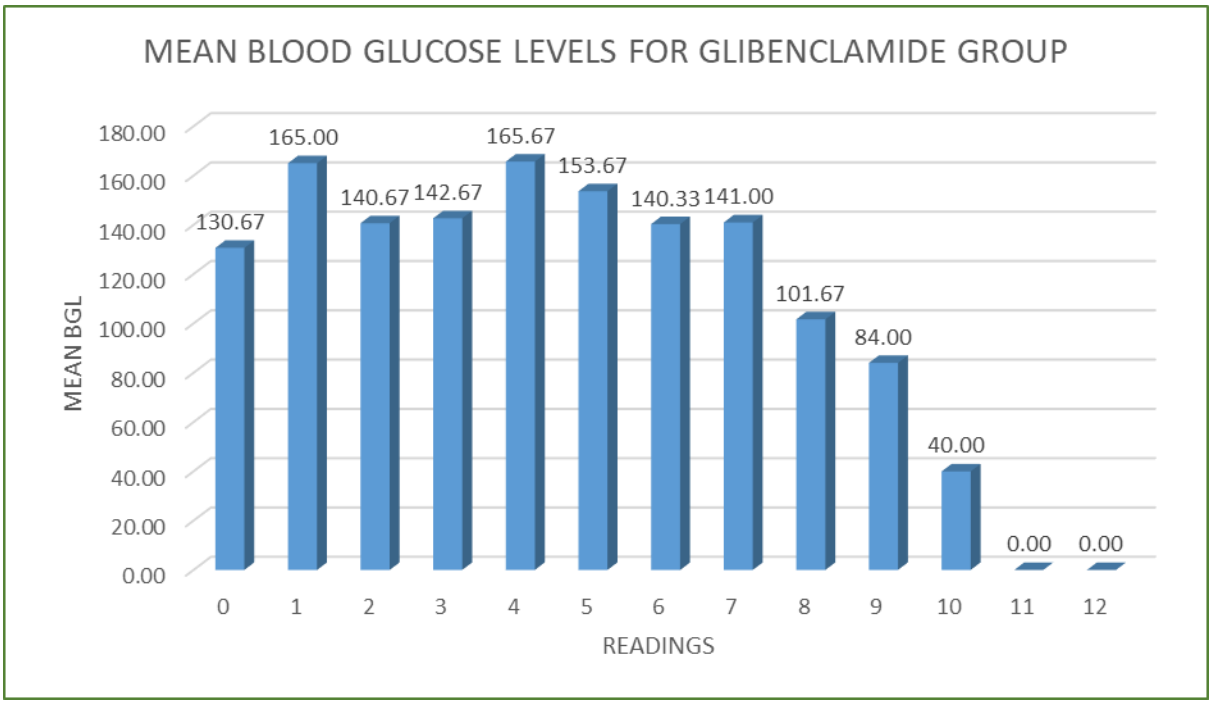
Table 2: Table showing the Blood Glucose Levels of Rabbits throughout experiment

	Group C (Sorrel Extract)				Group D (Jamun Extract)			
Readings	1	2	3	X	1	2	3	X
Initial	136	139	109	128 ±16.52	128	132	138	132.67 ±5.03
1st	140	171	141	150.67 ± 17.62	141	178	163	160.67 ± 18.61
2nd	154	121	152	142.33± 18.50	112	186	166	154.67 ±39.28
3rd	155	109	152	138.67 ± 25.74	128	185	218	177 ± 45.53
4th	176	130	127	144.33 ± 27.47	149	255	205	203 ±53.03
5th	155	119	141	138.33± 18.15	117	153	145	138.33 ± 18.9
6th	155	148	142	148.33 ± 6.51	164	125	128	139 ± 21.71
7th	152	119	143	138 ± 17.1	122	121	117	120 ± 2.65
8th	134	0	143	143 ± 92.3	0	0	0	0 ± 0
9th	157	0	152	103 ± 72.9	0	0	0	0 ±0
10th	147	0	124	90.33 ± 79.1	0	0	0	0 ±0
11th	130	0	125	85 ± 73.65	0	0	0	0 ± 0
12th	122	0	123	81 ± 70.7	0	0	0	0 ± 0

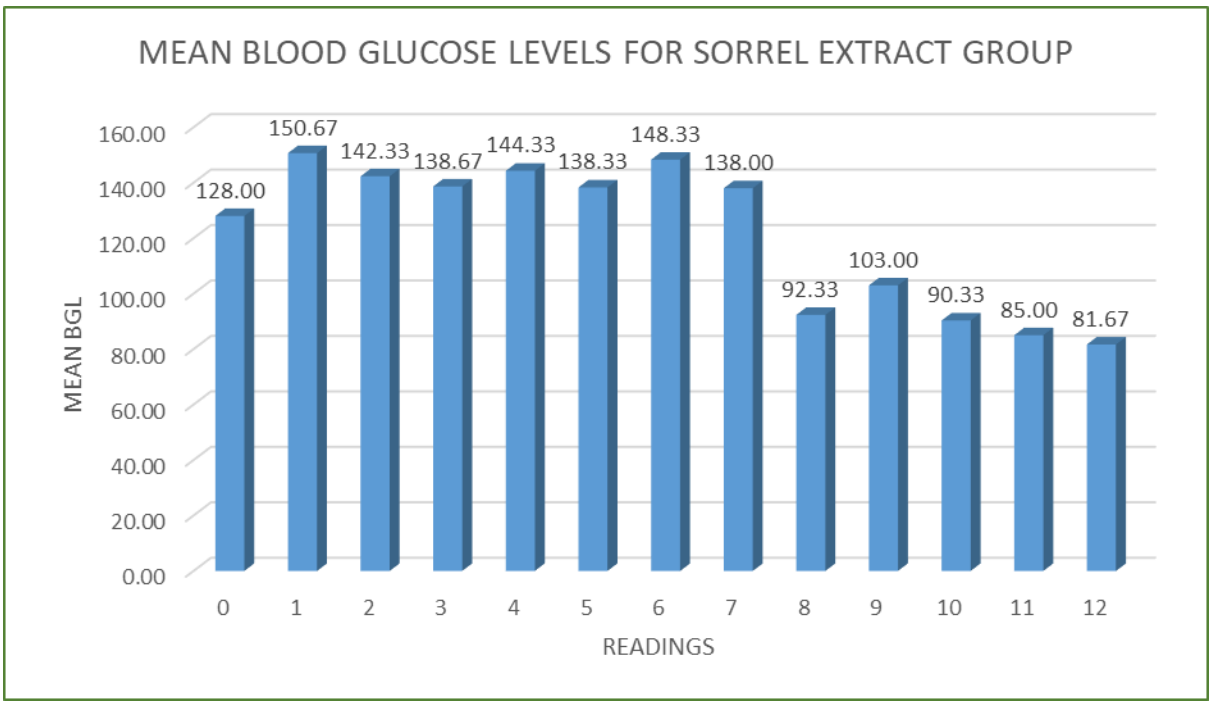
Graph 1: Mean Blood Glucose levels vs. Days for Control Group



Graph 2: Mean Blood Glucose levels vs. Days for Glibenclamide Group



Graph 3: Mean Blood Glucose levels vs. Days for Sorrel Extract Group



Graph 4: Mean Blood Glucose levels vs. Days for Jamun Extract Group

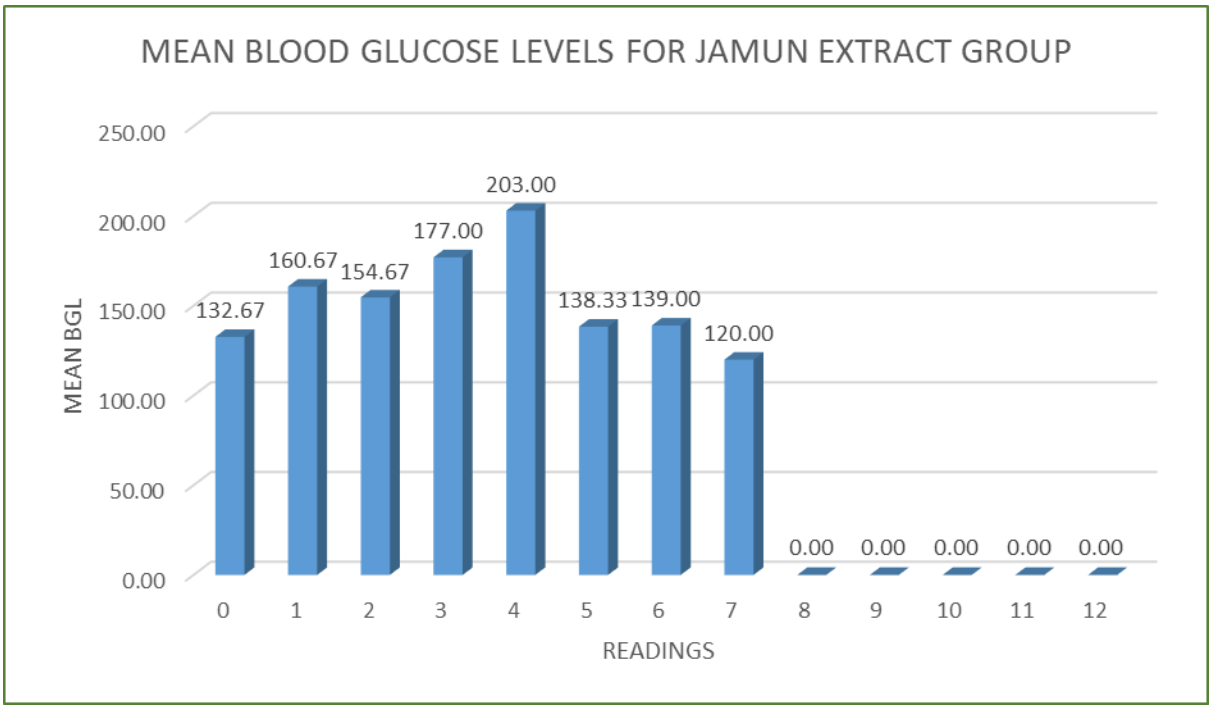


Table 3: One-way ANOVA for Group 2: Glibenclamide

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17419.44	2	8709.718	2.036115257	0.145299	3.259446
Within Groups	153994.2	36	4277.615			
Total	**171413.6**	**38**				

Table 4: Group 3: Extract 1 (*Rumex acetosa* - Sorrel)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	32546	2	16273	9.590651847	0.000458	3.259446
Within Groups	61083.23	36	1696.756			
Total	**93629.23**	**38**				

Table 5: Group 4: Extract 2 (*Syzygium cumini* L – Jamun)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3232.359	2	1616.179	0.238854969	0.788767	3.259446
Within Groups	243589.1	36	6766.363			
Total	**246821.4**	**38**				

Table 6: Two-way ANOVA for Glibenclamide versus Sorrel

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	42898.31624	12	3574.86	5.536408	0.002948	2.686637
Columns	1186.876068	1	1186.876	1.838123	0.200137	4.747225
Error	7748.401709	12	645.7001			
Total	**51833.59402**	**25**				

Table 7: Two-way ANOVA for Glibenclamide versus Jamun

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	109396.1	12	9116.338	10.95657	0.000109	2.686637
Columns	1246.154	1	1246.154	1.497704	0.244511	4.747225
Error	9984.513	12	832.0427			
Total	**120626.7**	**25**				

Figure 1: Multiple linear regression of Glibenclamide, Extract one and Extract two

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.965196758							
R Square	0.931604781							
Adjusted R Square	0.817925738							
Standard Error	2.108480309							
Observations	13							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	3	605.5431079	201.8477026	45.40302	9.25962E-06			
Residual	10	44.45689213	4.445689213					
Total	13	650						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
MEAN BGL (GROUP B)	-0.051240515	0.023064524	-2.221615917	0.050556	-0.10263148	0.0001504	-0.10263148	0.00015045
MEAN BGL (GROUP C)	0.132999916	0.016112621	8.254393547	8.94E-06	0.097098759	0.1689011	0.09709876	0.16890107
MEAN BGL (GROUP D)	-0.049519501	0.014545473	-3.404461421	0.006721	-0.08192883	-0.01711	-0.08192883	-0.01711017

Discussion For Experiment

Trends in Blood Glucose Levels of rabbits

Overall, there was a general decrease in the blood glucose levels after administration of Glibenclamide and extracts 1 and 2, as shown in **Table 1 and Table 2.0,** since the blood glucose levels of all the rabbits returned close to the baseline blood glucose levels taken before administration of Alloxan monohydrate. The blood glucose levels after the administration of Alloxan Monohydrate ranged from 131 – 202 mg/dL and these values were significantly reduced after administration of Glibenclamide, Extract 1(*Rumex acetosa*/Sorrel) and Extract 2 (*Syzygium cumini*/Jamun) to values ranging from 116 – 123 mg/dL. However, during the course of the experiment, the blood glucose levels were way above normal even though hypoglycemic agents were being administered to the rabbits. Possible reasons for this include the sugar content of the diet of the rabbits, the frequency of feeding and potentially the dose of the hypoglycemic agents being too low.

Rabbit pellets, as the one given to the rabbits used in this experiment, usually contains beet pulp used to aid in digestion. Beet pulp has 50% fibre and has a high water holding capacity along with a high caecal retention time. It is used mainly as an energy concentrate since it has a high content of digestible fibre, pectins and sugars and it has a low starch content. The pellet used contains 7.8% of sugars, therefore, it can potentially increase glucose levels in the body **[32]**.

Also, the rabbits food containers were being refilled in the morning and in the night and the containers were placed in the cages without being removed so the rabbits had continuous access to the pellets. Therefore, continuous eating could have potentially caused an increase in the blood glucose levels due to the sugar content of the pellets.

The weights of the rabbits were increased from the weights taken initially since the rabbits were growing from kittens into adulthood and also because they were constantly being fed.

ANOVA statistics were performed to determine if there were any significant differences of the Reference drug (Glibenclamide), Extract 1 (*Rumex acetosa* - sorrel) and Extract 2 (*Syzygium cumini* L – Jamun) on the concentration of blood glucose level between and within groups over a time period of twelve days. **Table 3** shows the one-way ANOVA result, between and within group 2, the Glibenclamide treated group which indicate that there was not a statistically significant difference in the concentration of blood glucose level of Glibenclamide group between at least two groups (F (2, 36) = [2.036], P = 0.145). This indicates that Glibenclamide is an effective antidiabetic agent to lowered blood glucose. Glibenclamide was given from the 25th March, 2022 to 29th March, 2022, at a dose of 2.5 mg/kg, which has the potential of lowering blood glucose levels back to normal since it is one of the most potent anti-diabetic medications[33]. However, 2.5mg/kg did not lower the blood glucose levels as expected, therefore from the 31st March, 2022 to the last day of the experiment, 5 mg of Glibenclamide was administered

regardless of the weight of the rabbit and this led to a decrease in the blood glucose levels back to normal.

The results of **Table 4**, i.e, between and within group 3, reveals that there was a statistically significant difference in the concentration of blood glucose level of sorrel group between at least two groups (F (2, 36) = [9.591], P = 0.000458). This indicates that this extract will have a different potency in lowering blood glucose. In the *Rumex* genus, there are many isolated constituents such as lignans, tannins, anthraquinones, flavonoids, terpenes, etc., which are associated with antibacterial, antitumor, anti-inflammatory and anti-diabetic properties. Aqueous extract from the leaves of the *R. acetosa*/Sorrel plant was given at a dose of 6mL/kg from the 25th March to 29th March. The dose was then increased by 1 mL from 31st March to 2nd April. On 4th April, the dose was reduced to just 1 mL due to the dose being considered too high. It was increased to 1.5 mL from 6th April – 8th April then increased further to 2 mL on 10th April – 14th April, which lead to the reduction of the blood glucose levels to normal.

In between and within results as shown in **Table 5**, revealed that there was not a statistically significant difference in the concentration of blood glucose level of Jamun group between at least two groups (F (2,36) = [0.239], P = 0.79). This indicates that Jamun has a statistically significant potency in lowering blood glucose level. In terms of the *Syzygium cumini*/Jamun plant, the gallic, catechin, ferulic, ellagic and quercetin constituents of the different parts of the plant are thought to be α-amylase inhibitors. The enzyme, α-amylase is used in the body to convert dietary starch into maltose, maltotriose and oligoglucans. These products are then converted into glucose by the enzyme, α-glucosidase. The constituents of the Jamun plant inhibits α-amylase, which prevents dietary starch conversion, therefore glucose would not be made. This is the proposed mechanism as to how the Jamun plant controls hyperglycemia[34]. At the beginning of the experiment, Jamun leaf extract was being given at a dose of 6mL/kg from 25th March, 2022 to 29th March, 2022. The dose was then increased by 1 mL from 31st March to 2nd April. However, on 4th April, blood glucose levels were close to normal so just 1 mL was given so as to prevent hypoglycemia.

Table 6 shows the results of a two-way ANOVA which was performed to analyze if Sorrel extract will have antidiabetic activity compared to Glibenclamide. However, a two way ANOVA reveal that there was not a statistically significant difference between the antidiabetic effects of Glibenclamide and Sorrel extract. P = 0.20 (P value greater than 0.05). The results show that there were no statistical differences found in the effectiveness of both Glibenclamide and Sorrel extract. This indicates that *Rumex acetosa*/Sorrel is as potent as Glibenclamide as a hypoglycemic agent.

Table 7 shows the results of a two-way ANOVA which was performed to analyze if Jamun extract will have antidiabetic activity compared to Glibenclamide. However, a two way ANOVA reveal that there was not a statistically significant difference between the antidiabetic effects of Glibenclamide and Jamun extract. P = 0.245 (P value greater than 0.05). This indicates that there were no statistical differences found in the effectiveness of both Glibenclamide and Jamun extract.

Based on the analyses done on Glibenclamide versus Sorrel and Glibenclmide versus Jamun, the results indicate that both sorrel and Jamun fruit extract will have a positive outcome in improving (lower) the concentration of blood glucose in Alloxan Monohydrate-induced diabetic rabbits when compared to Glibenclamide. The Null hypothesis was therefore rejected and alternative hypothesis was accepted for both Glibenclamide versus Sorrel and Glibenclmide versus Jamun. These results are shown in graph 1 -graph 4.

Figure 1: Multiple linear regression was performed to test if Glibenclamide, extract 1, and extract 2 will significantly predict concentration of blood glucose level. The fitted regression model was Type II Diabetes Mellitus = (-0.0512) + 0.133 – 0.0495). It was found that Glibenclamide, sorrel, and Jamun significantly lowered the concentration of blood glucose level (R^2 = 0.932, F (3, 10) = -0.0512, P = 0.05; 0.133, P = 8.94184E-06; -0.049519501, P = 0.00672051 respectively).

Figure 1: Multiple linear regression of Glibenclamide, Extract one and Extract two

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.965196758							
R Square	0.931604781							
Adjusted R Square	0.817925738							
Standard Error	2.108480309							
Observations	13							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	3	605.5431079	201.8477026	45.40302	9.25962E-06			
Residual	10	44.45689213	4.445689213					
Total	13	650						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
MEAN BGL (GROUP B)	-0.051240515	0.023064524	-2.221615917	0.050556	-0.10263148	0.0001504	-0.10263148	0.00015045
MEAN BGL (GROUP C)	0.132999916	0.016112621	8.254393547	8.94E-06	0.097098759	0.1689011	0.09709876	0.16890107
MEAN BGL (GROUP D)	-0.049519501	0.014545473	-3.404461421	0.006721	-0.08192883	-0.01711	-0.08192883	-0.01711017

Conclusions

Glibenclamide, *Syzygium cumini*/Jamun plant and *R. acetosa*/Sorrel plant all showed potent anti-diabetic properties. Glibenclamide given at a dose of 5 mg, *Syzygium cumini*/Jamun leaf extract given at a dose of approximately 6.5 mL/kg and *R. acetosa*/Sorrel leaf extract given at a dose of approximately 6.5 mL/kg can be used to reduce blood glucose levels in Alloxan Monohydrate-induced hyperglycemic rabbits over a period of 21 days. Therefore, Null hypotheses 1 (a), 2 (a) and 4 (a) were rejected due to results from ANOVA analyses, meaning that Rumex acetosa/Sorrel and Syzygium cumini/Jamun leaf extracts were effective in lowering blood glucose levels in Alloxan Monohydrate-induced rabbits as Glibenclamide, the response to the aqueous extracts were concentration dependent and the extracts were eligible for commercialization, while Null hypothesis 3 (a) was accepted, meaning that the aqueous extracts were not toxic to the rabbits.

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