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Therapeutic and Scientific Validation of Divine Fermented Mild Acidic Polyherbal Mixture Therapy for the Cure of Diabetes Type I&II

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ABSTRACT

Purpose: following the unprecedented exploration of natural products including organisms (plants, animals or microorganisms) have been shown to possess health benefit for animals and humans. According to the estimation of the (WHO), in developing countries 80% of the population are still depending on traditional medicine or folk which are mostly prepared from the plant to prevention or treatment of diabetes type 1& type 2. Methods: Reposing of the selection, characterization of bacteria through traditional, phytochemical and microbial fermentation methods base on antidiabetic type 1 & type2 drugs discovery. Thus, these approaches and techniques is essential to provides a better lead way otherwise. Taking a bold step of faith, to approach the techniques for diabetes type1 & type 2. Results: the results for microbiological, screening of bacteria reveals that Bacillus sp (spore former), motile, rod (+) was spotted to have the highest strength of mutational properties for drug discovery followed by Pseudomonas sp alongside with Enterobacter have always been maintaining the order of drugs alternative (fermentation products). Other strength of species which have proven to have less mutant for the battle were also acknowledged too namely: Serratia sp, Streptococcus sp and Citrobacter freundii. Further investigations were also observed on the tolerance level of each respected organism on their mutational properties on pH and Temperature. It was observed that Bacillus sp and Pseudomonas sp performed the highest strength of mutant properties both at mesophilic range 35^{O}C or 40^{O}C and alkalophilic range pH 7 -8(basification). Having buffered from mild alkaline to neutrality then the acidified bacteria take up the lead by utilizing the enzyme protease to enzyme amylase at mild acidic pH 5-6.7 with fewer specialized bacteria namely. Serratia sp, Streptococcus sp and Citrobacter freundii. Phytochemical screening of different plants species both on qualitative and quantitative analyses were employed using Beta vulgaris (beet root), Ziniber officinale(zinger), Moringa leaf/seed (Moringa oleifera), (Comellia sinensis)green tea), Bitter cola(Garcinia Kola), (Mentha) Mint, Tomato(Solanum lycopersicum), Turmeric(Curcuma longa), etc., respectively contain all the natural antioxidant, bioactive compounds, vitamins as well as an appreciable amount of micronutrient/ macronutrient for combatting diabetes Type 1&2. Results of both qualitative and quantitative analyses with regards to different solvents were noted, the universal solvent almost have taken the led with dense (+++) followed by methanol and least of all was accentuated from acetone with less (+) or -. For quantitative analysis the investigations were meticulously and duly calculated based on their percentage's differences are also noted too. Furthermore, the clinical trials from different local governments areas in Kaduna State like those of Kachia, Igabi, Zangon Kataf, Kubau, Kaura Sabon Gari, etc., respectively have shown that poly herbal mixed culture created a greater vacuum for the reduction of both high blood pressure and glucose level to the bare -rest minimal level of isotonic point. Thus, the interesting results is not only for pharmaceutical and medical importance without an oater level of qualm as well as other ailments alone but also for humanity.

In view of the current medication of diabetes type 1&2 on controlling and lowering blood glucose level in the vessel to isotonic points. However, most mordant drugs have many sides effect causing some serious medical problem during a period of treatment. Therefore, traditional medicine has been used for a long time and play an important role as alternative medicine. Traditional medicine performed a good clinical practice and is showing a bright future in the diabetes type1 &type 2.

Key words; *Traditional (isolation of bacteria) Phytochemical screening; Antidiabetic; drugs discovery (poly herbal mixed cultured capsule)*

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I. INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid, and protein metabolism caused by insulin deficiency, often combined with insulin resistance. It is considered one of the five leading causes of death in the world. About 150 million people are suffering from diabetes worldwide and it is almost five times more than the estimated ten years ago, which may be doubled by 2030 **[1].** Further, the International Diabetes Federation predicts that by 2045 the number of individuals effected with diabetes will increase to 629 million [2]**[2].** It is projected that the total global economic burden will escalate from the U.S. $ 1.3 trillion in 2015 to $ 2.5 trillion in 2030, which represents a staggering increase in costs as a share of global GDP from 1.8% in 2015 to 2.2% in 2030 **[3].** Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, the search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal medicines with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine. The plants provide a potential source of hypoglycemic drugs because many plants and plant-derived compounds have been used to treat diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes, and reports are evident in numerous scientific journals. Ayurveda and other traditional medicinal systems for the treatment of diabetes describes numerous plants used as herbal drugs. Hence, they play an indispensable role as an alternative medicine due to fewer side effects and low cost. The active principles present in medicinal plants have been reported to possess the pancreatic beta cells re-generating, insulin-releasing, and fighting insulin resistance. Hyperglycemia is involved in the etiology of the development of diabetic complications. Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from the intestine and glucose production from the liver **[4].** The currently available drugs in the management of DM are Insulin and oral hypoglycemic, with their glycemic efficacy, mechanism of action, and side effects. Still, there is a quest to develop more effective antidiabetic agents **[5].**

Diabetes mellitus is a non-infectious endocrine disorder which is characterized by the disturbance in metabolism of carbohydrate and associated with hypoglycemia. It is linked with developing of various serious diseases like micro vascular (nephropathy, retinopathy, nephropathy) and macro vascular (peripheral vascular disease and coronary heart diseases) **[6].** Diabetes mellitus also known as diabetes which was observed as diseases related with "sweet urine" and muscle loss. Glucose blood levels are maintained by insulin which is a hormone released from the pancreas. When these level increases, insulin is produced from the pancreas and maintained the level of glucose. In diabetic patients, the production of insulin is absent or less which causes hyperglycemia **[7].** Diabetes mellitus are three types Type 1, Type 2 and gestational diabetes mellitus. Type 1 Diabetes mellitus is known as insulin dependent diabetes mellitus which is due to total loss of function of β cell of islets of Langerhans which are present in pancreas. Type 2 Diabetes mellitus is known as insulin non dependent diabetes mellitus which is temporary loss of β cell mass and it is due to genetic predisposition and mostly occur in obese persons and associated with high blood pressure and high cholesterol levels. The aim of treatment of type 2 diabetes mellitus is decreases the insulin resistance and increases insulin secretion. Gestational diabetes is a type of diabetes which present with hyperglycemia in pregnant women. It usually appears in 2-4% pregnancies in 2nd or 3rd trimester **[8].** The symptoms of diabetes mellitus are poly dipsea, polyuria, poly phagia, fatigue, nausea, vomiting, impotence in men, slow healing wound and blurred vision. According to International Diabetes Federation (IDF) survey in 2016 diabetes is a disorder which affects 415 million people in the world and it may increase to 642 million by the year 2040 **[9].** According to Aroma world reports 61.3 million people have diabetes in INDIA and consist of 20-79 age group in the population. It may approximately be doubled by the year 2030.

For instant, Indian is also known as diabetes capital of the world and affects mainly rural and urban people **[10].** The frequency of diabetes is progressively increases in urban areas of India. The frequency of diabetes in urban area abstract diabetes mellitus is becoming a common metabolic disorder which has serious threat to public health in the world. There are chemicals and biochemical agent that helps in controlling diabetes but there is no permanent remedy available which helps to get recovered completely from this disorder. By conducting large number of research work, numerous traditional medicines have been found for diabetes permanent cure. Substances and extracts isolated from different natural resources especially plants have always been a rich arsenal for controlling and treating diabetes problem and complication arising due to it. So, this review helps the reader to understand the importance of various types of herbal and poly herbal formulations present traditionally which can be used to treat diabetes mellitus. Rural population. Decreased exercise, increasing weight and tension, change in diet, malnutrition, alcohol consumption, viral infection like those of

HIV/ AIDS, covid-19(recently) are the major causes of diabetes mellitus in last 40 years as well as two years ago **[11].** Female diabetic patients are more than compared to male diabetic patients because hormone and inflammation act differently in women. The people who are less educated have numerous diabetes disorder in comparison to the educated people. The utmost percentages of people having diabetes are lives in developing countries **[12]**

1.1 **RESEARCH PROBLEM**

. The frequency of diabetes worldwide abstract diabetes mellitus is becoming a common metabolic disorder which has serious threat to public health in the world. There are chemicals and biochemical agent that helps in controlling diabetes but there is no permanent remedy available which helps to get recovered completely from this disorder. For instant let gear our minds toward simple definition of Diabetes which refers to a group of condition characterized by high blood glucose commonly referred to as glucose sugar. Too much sugar (Carbohydrate/synthetic sugar) in the blood can cause serous ill-health and sometime life threatening.
There are two types of chronic diabatic condition:
Type 1 diabates
Type2 diabates
Pregnant women may have gestational diabetes
Prediabetes is when the blood sugar level is at the borderline higher than normal but lower than diabetes. Prediabetes may or may not progress to diabetes
During food digestion, carbohydrate or carb- break down into glucose which is carried by the blood stream to various organs of the body. Thus, the presence of insulin which are hormone produced by beta cells of the pen crease and it is

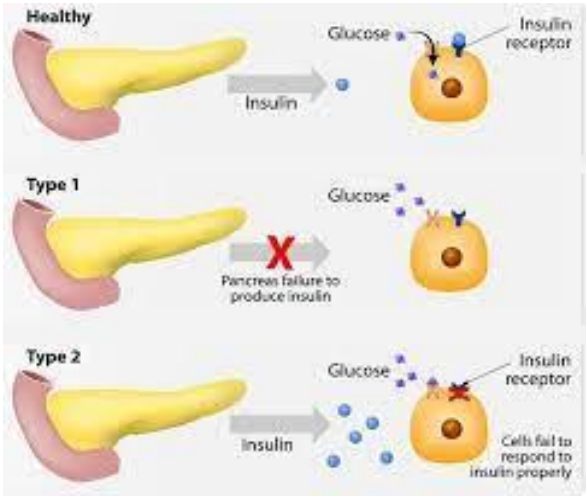

Fig1. Posted Healthy Pancreas and Pancreas types1 and type2 diabates

necessary for glucose intake by the target cells. In a non-diabetes patient (Normal), the beta cells of the pen crease produce insulin; insulin binds to the its receptor on target cells and induce intake of minimal or an appreciable amount of glucose (Nature design). At this particular point in time the genetic inherit ant are not altered by viruses (HIV/AID) which have ravage people lives to their early grave for over 40years as well as covid-19/Omicron for just two years or thereafter. In Type 1 diabetes: In type I diabetes the beta cells of the pen crease were destroyed by viral infectious/ immune system; thus, the insulin production has little or no effort for circulation within the respected organs/tissues of concern. To this effect the insulin binds to the receptor on target cells in reciprocal less glucose is taken into the cells, more or abundant of glucose were found wanting inside the blood helplessly. Type1 diabetes is characterized by early on set symptom commonly start suddenly and before the age of 20 years. One could imagine a young man or lady have diabatic at such a tender age, the resultant effect to these sudden life-threatening illnesses is due to illegal sex rampantly. Type 1 diabetes is normally managed with insulin till dead (Dead warrant is already signed through illegal sex). Furthermore, type 1 diabetes are insulin dependent for one's life time till dead. Similarly, to Type2 diabetes but there is a bit different. In type 2 diabetes the pen crease produces enough insulin but something goes wrong either with the receptor binding insulin signaling inside the target cells. The cells are not responding to insulin and therefore cannot be import glucose; thus, glucose remain helpless in the blood. On the contrary type 2 diabetes are insulin resistant; here again genetic factor predispose susceptibility to the disease (human genetic DNA is altered by the HIV/AID/Covid-19). Thus, Both Antiretroviral drugs/ artificial vaccine is rendered helplessly to permanent

cure. Once life style to Type2 diabetes plays a very important role for keeping one update (high blood rises and lowered by maintaining the clinical algorithm till death). Typically, obesity, inactive life style and unhealthy diet are associated with higher risk of type 2 diabetes. Type2 diabetes is characterized by adult on set. Symptom usually appeared gradually and start after the age of 30. Type 2 diabetes account for about 80 to 90% of all diabetic's management focuses on weight loss and include a local diet. Symptom of type 2 diabetes, frequent urination, excessive thirst, weakness, loss of sigh, nerve damage, kidney disease, heart disease, stroke, poor blood circulation, high intake of antiretroviral drugs (HIV/AIDS) etc. respectively

1.2 JUSTIFICATION/RATIONAL OF THIS RESEACH

The long -term complications of uncontrolled diabetes are well known- damage to eyes, kidneys nerves, blood vessels and other organelles. Most of these damages are attributed to chronic elevated glucose sugar. Both type 1 and 2 diabetics and prediabetics are aware of the dangers and sometimes consider applying herbal. Health overcomes e.g., life expectancy, quality of life and patient satisfaction have not been steadily improved by the conventional medicine which are prone to be highly expensive to the reach of a common man. Thus, there are many unfulfilled health expectations and issues with regard to maintaining wellness and treating several chronic, irreversible, or incurable disease using the conventional methods. In recent years the used of herbal medicine have been recognized as a potential alternative to the existing technologies for cure of diabetes patients in comparison to the orthodox medicine which are highly expensive to the common man once again.

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants **[13].** There has been a gradual increase in use of alternative therapy worldwide. A survey released in May 2004 by the National Center for Complementary and Alternative Medicine focused on who used complementary and alternative medicines (CAM), what was used, and why it was used. The survey was limited to adults, aged 18 years and over in the year 2002, living in the United States. According to this survey, herbal therapy, or use of natural products other than vitamins and minerals, was the most commonly used CAM therapy (18.9%) when all use of prayer was excluded **[14].** In the United Kingdom, it is estimated that in 1996 alone, at least 72 million pounds was spent on alternative therapies (licensed herbal medicine, homeopathic remedies and essential oils for aromatherapy). Herbal remedies are very common in Europe. In Germany, herbal medications are dispensed by apothecaries (e.g., Apotheke). Prescription drugs are sold alongside essential oils, herbal extract, or herbal teas. Herbal remedies are seen by some as a treatment to be preferred to pure medical compounds which have been industrially produced. In the UK, herbal remedies that are bought over the counter are

Furthermore, the increasing cost of using the existing technologies for orthodox medicine as therapy for the treatment of diabetic mellitus, in reciprocate using available plants materials is increasingly being considered. Thus, the use of plant like those Aloe-vera., Better melon, Gallic, green tea leaves, moringa leave etc. respectively as materials for substitution of expensive orthodox medicine not easily available is quietly and essentially a welcome development or novelty. The results of these research project will be duly highlighted of the significance of herbal plants using locally available plants as an efficient means of treating diabetes mellitus present in hypertensive patients.

1.3 SCOPE OF THE STUDY

Diabetes mellitus (DM) is one of the most common metabolic diseases; it is known as an increase of the blood glucose level and impaired metabolism of proteins and lipids. Currently, diabetes is considered as one of the most critical issues in the world. Much research performed more efforts to seek new natural antioxidant molecules, which are considered to be relatively safer and with less or without side effects. Recently, the attention is focused on plants which contain high concentrations of phytochemical compounds due to their potential health-promoting effects. The aim is to establish a prototype consortium of herbal potential antidiabetic and antioxidant effects using *series or combination of plant* extracts for the cure of Diabetes mellitus in human with little or no side effect

1.4 RESEARCH QUESTION

- Is the validity of mild acidic herbal medicine curable?
- Does the efficacy of herbal dosage guarantee the safety of the patient?
- What is the fate and health of peoples who have ingested the by- product of synthetic sugar or chemical fertilizer?

- Does herbal medicine for diabetic patient be taken without habitually or acquainted in doing exercises which is just the cheapest way? (Is no buying the car that matters but the maintenance culture)
- That will be the market price of mild acid herbal medicine in comparison to NGO therapy
- Will there be an appreciable reaction to people who takes the mild acid herbal medicine with ease or grumbling?
- Does the mild acid herbal medicine neutralize genetical heredity of sickle cell diabatic patient as people always said it genetical heredity from one family?
- If there could be a breakthrough in the mild acid herbal medicine; What will be the managerial set up for the price high/ low or does the demand of mild alkaline herbal medicine by diabetic altered the change in market price by the management?
- If the demand of mild acid herbal medicine is high will the management encourages dilution so as to scare their costumers?

1.5 RESEAECH AIM AND OJECTIVES

The main purpose of this study is to assess the curable herbal medicine for diabetes Mellitus

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The Specific Objectives of This Study Are:

To evaluate the phytochemical screening of the herbal plant

To assess any opportunistic bacteria in the extract

To determine the consistency viability of herbal plant

To explore how patient are newly diagnosed with types I&II diabetes

II. LITERATURE REVIEW

Relationship between HIV and Diabetes Mellitus (DM)

The unequivocal success of antiretroviral therapy (ART) in controlling HIV replication and restoring immunity has been tempered by the recognition that metabolic diseases, such as diabetes mellitus (DM), are increasing in incidence among people living with HIV. Studies from high-income countries have reported that the incidence of diabetes in HIV-infected adults receiving ART is between 1% and 10%.**[15]** Conventional risk factors, such as obesity, ageing and male sex, are important determinants of diabetes. [However, specific antiretrovirals (ARVs) and ARV-related weight gain and lipodystrophy are recognized risk factors. High pre-ART viral loads and low baseline CD4+ counts may also increase the risk of insulin resistance and accelerate the pathogenesis of diabetes. The association between HIV infection and DM in sub-Saharan Africa (SSA) has not been well documented. **[16]** While diabetes incidence is increasing in the general population in southern Africa, there is very little describing the incidence and determinants of diabetes among HIV-infected adults in this region.

Furthermore, with the advent of highly active antiretroviral therapy (HAART), human immunodeficiency virus (HIV) infection has become a manageable chronic medical condition; however, the impact of metabolic complications related to long-term exposure to HAART and aging on the long-term successful management of HIV infection remains to be monitored and investigated [17]. It is observed that the risk of diabetes mellitus (DM) among HIV-infected patients has increased with long-term exposure to HAART. DM will subsequently increase the risks of coronary artery disease, stroke, peripheral vascular disease, retinopathy, chronic kidney disease, and dementia. Previous studies hypothesized that interactions between HIV infection, HAART, and inflammation could significantly contribute to the risk of DM**,[18]** moreover, older age, male gender, and hepatitis C virus (HCV) co-infection were also identified to be related to development of DM.

2.1 EPIDEMIOLOGY AND RISK FACTORS FOR DIABETES IN PLWH

Epidemiology

PLWH have a unique set of risk factors that increases their likelihood of developing diabetes. Illustrating the greater burden of diabetes in PLWH on ART, a study from 2005 on the Multicenter AIDS Cohort Study (MACS) of gay and bisexual men with (HIV+) and without HIV (HIV-) found that the incidence of diabetes was found to be more than four-fold higher in HIV+ men and that the prevalence of diabetes was 14% in HIV+ men on ART and 7% in HIV+ men not on ART, compared to 5% in HIV- men. These differences were significant even after adjusting for age and body mass index (BMI). Of note, the majority of HIV+ men on ART in the study were on first generation protease inhibitor (PI) therapy (discussed further in the section Protease Inhibitors) [18]. Other studies have described incidence rates of diabetes in PLWH of 4.4 cases per 1000 person-years of follow-up in the Swiss HIV Cohort Study and 5.72 cases per 1000 person-years of follow-up in the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study of participants from Europe, the US, Australia, and Argentina **[19].**

Plate1. Posted pure culture of Klebsiella sp grown in home base solid agar medium

The effect of HIV disease on developing diabetes is also growing in low- and middle-income countries (LMIC), where the majority of the people with diabetes live **[20].** Prevalence estimates of diabetes in PLWH in LMIC range from 6.8% in Chile to 26% in Cameroon. PLWH and diabetes in LMIC are younger than those in high income countries. The wide range of prevalence estimates of diabetes in PLWH in LMIC may be in part because of differences in the definitions used to identifying diabetes. In addition to factors common to PLWH globally, urbanization may be a contributing factor to the development of diabetes in PLWH in LMIC **[21].**

2.2 RELATIONSHIP BETWEEN COVID-19 AND DIABATES

T he observed association between diabetes and covid-19 might be attributed to the effects of sars-cov-2 infection on organ systems involved in diabetes risk. Covid-19 might lead to diabetes through direct attack of pancreatic cells expressing angiotensin converting enzyme 2 receptors, through stress hyperglycemia resulting from the cytokine storm and alterations in glucose metabolism caused by infection, or through precipitation of prediabetes to diabetes **[22].** A percentage of these new diabetes cases likely occurred in persons with prediabetes, which occurs in one in five adolescents in the United States. †††† steroid treatment during hospitalization might lead to transient hyperglycemia; however, only 1.5%–2.2% of diabetes codes were for drug- or chemical induced diabetes, with the majority of codes being for type 1 or type 2 diabetes. Alternatively, covid-19 might have indirectly increased diabetes risk through pandemic-associated increases in body mass index, §§§§ a risk factor for both serious covid-19 illness and diabetes. Future studies addressing the role of comorbidities and increases in body mass index in post–covid-19 diabetes are warranted. Although this study can provide information on the risk for diabetes following sars-cov-2 infection, additional data are needed to understand underlying pathogenic mechanisms, either those caused by sars-cov-2 infection itself or resulting from treatments, and whether a covid-19–associated with diabetes diagnosis is transient or leads to a chronic condition. Evidence of increased pediatric type 1 diabetes has been reported during the covid-19 pandemic **[23].** However, the observed association of increased risk for diabetes diagnosis following SAR-Cov-2 infection would not be explained solely by delayed case covid-19 which has disproportionately affected racial/ethnic minority group and these above 18 years in these group are also at increased risk for type 2 diabetes **[24].** An association between covid-19 and the new pediatric diabetes diagnosis might disproportionally affect racial/ethnic minority group. Race/ethnicity data were un available in the present data set; however, future studies should be solely addressed regarding to racial and ethnic disparities in covid-19 and diabetes. Thus, whether person age above 18 years who might have been at risk of covid-19 as well as the risk for delaying health care **[25]**

2.3 DIAGNOSIS OF DIABETES MELLITUS

The diabetes can be measured by analyzing the blood sugar levels. The blood sugar level in healthy man on fasting are 80 mg/dl and in postprandial state is up to 160 mg/dl. Different test for diagnosed of diabetes in laboratory are finger prick blood sugar test, fasting blood sugar, glucose tolerance diagnostic test, glycohemoglobin **[26].**

Plates 2. posted mixture of Bacillus sp, Enterobacteria sp Citrobacter sp

2.4 PATHOPHYSIOLOGY OF DIABETES MELLITUS.

The main role in pathophysiology of diabetes is oxidative stress. The imbalance between production of reactive oxygen species (ROS) and capacity of enzymatic or non-enzymatic antioxidant are known as oxidative stress. Reactive oxygen species contains free radicals such as super oxide, hydroxyl, peroxyl, hydroperoxyl and non-radical species such as hydrogen peroxide. Antioxidant contains super oxide dismutase, glutathione reductase, vitamins A, C and E, carotenoid, glutathione and trace elements. Low density lipoprotein cholesterol is oxidized in the presence of reactive oxygen species which taken up by hunter receptor in scavenger cell and cause formation of foam cells and arterial sclerosis plaques. These ROS can Stimulate various damaging pathway which have important role in growth of diabetes disease. Some important pathways are glucosamine pathway, sorbitol aldose reductase pathway, electron transport chain, protein kinase C stimulation. Stimulation of these pathways and mode of action can lead to atherosclerosis, programmed cell death, lipid per oxidation, advanced glycation end product (Ages) formation, amylin and failure of pancreatic β cell function. It is proven that sequence specific DNA binding factor (nuclear factor erythroid derived 2 like 2) along with their negative regulator (kelch like ECH associated protein 1) have important cell protection mode of action against oxidative stress **[27].**

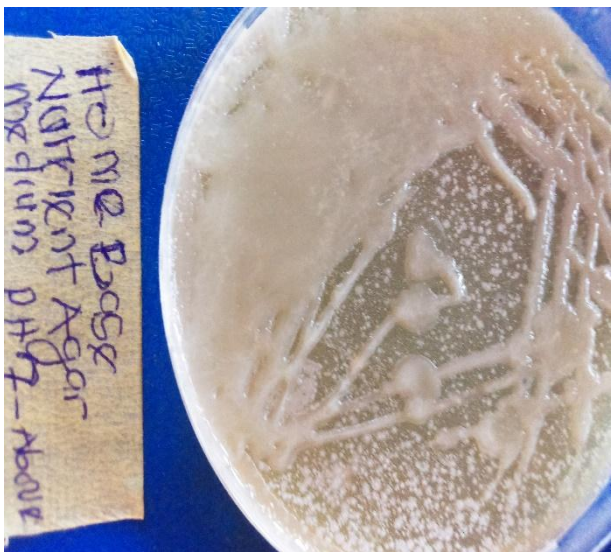

Plate3. Posts mixed culture of Klebsiella sp and others using home base solid agar medium

2.5 ANTIDIABETIC DRUGS

Antidiabetic drugs Diabetes mellitus disease can be prevented by regulating the blood sugar level with various types of medicines, acquiring to different exercise or yoga therapy or diet plan [9]. Currently available therapies of diabetes mellitus are insulin treatment for type 1 diabetes mellitus and other oral hypoglycemic

drugs such as sulphonyl-urease, thiazolidinediones, peptide analogs for treatment of type 2 diabetes mellitus **[28].**

2.6 HERBAL REMEDY

Treatment of Diabetes mellitus without any adverse effects is still the biggest question to medical practitioners. According to world ethano-botanical 800 medicinal plants are used for the prevention of diabetes mellitus. Clinically proven that only 450 medicinal plants possess anti diabetic properties from which 109 medicinal plants have complete mode of action. In ancient time doctor and lay person used traditional medicinal plants with their active constituents and properties for the treatment of various diseases such as heart diseases, cancer and diabetes. There is a long history of traditional plants used for the control of diabetes in India and China. There are various books available such as Charaka Samhita and Susruta Samhita which explains phytopharmacology features of diabetes and its adverse effect **[29].** Synthetic drugs which are used for treatment of diabetes are associated with various adverse effect such as sickness, vomiting, dysentery, alcohol flush, migraine, swelling, malignant anemia and faintness. Herbal drugs are proved to be a better choice over synthetic drugs because of less side effects and adverse effects. Herbal formulations are easily available without prescription. These herbal drugs are used for life threatening disease. These drugs are also used when chemical drugs are ineffective in treatment of disease. These are natural and safe drugs i.e., there is no toxic effects. Herbal drugs permanently cure person and treat the disease while synthetic drugs are not permanently cured the diseases. Herbal formulations contain natural herbs and fruits and vegetables extract which are beneficial in treatment of various diseases without any adverse effects. On the other hand, chemical drugs are prepared synthetically and have side effect also. Herbal formulations are cheap as compared to all opathic medicines. Herbal formulations are Eco friendly. Herbal formulations are produced from natural products while all opathic medicines are produced from chemical and chemically modified natural products. Herbal formulations are available without prescription while all opathic medicines are available with prescription **[30].**

2.7 RELEVANT CLINICAL TRIALS WITH MEDICINAL PLANTS AND NATURAL PRODUCTS

The number of clinical trials carried out with medicinal plants as antidiabetic agents are quite limited and some of them are developed with herbal formulation **[31].** Other studies include specific medicinal plants or extracts, and measure some characteristic parameter, such as glycated hemoglobin (HbA1c) in patients with T2DM **[32].** However, all of these studies were of poor quality with unclear methods of randomization, threats to blinding, and lack of baseline demographics. In recent years, some interesting articles on natural antidiabetics, including medicinal plants used in folk medicine and phytotherapy, have been reported. Among these plants, bitter melon (M. charantia), nettle (U. dioica), sage (S. officinalis), and walnut tree (J. regia) are widely used in folk medicine and some clinical studies were also developed in-situ

Currently the medicinal plants and herbs are being used in extract forms into drugs discoveries(products) for their anti-diabetic activity. Various clinical studies confirmed that medicinal plants extracts and drugs discoveries products showed anti-diabetic activity and restoring the action of pancreatic β-cells **[33].**

Aloe

[34] reviewed antidiabetic properties of Aloe barbadensis Mill., syn: Aloe vera (L.) Burm.f., (Aloaceae) and selected a study with 72 diabetic women without drug therapy, divided into two groups. They received A. vera gel (15 g) or placebo for 42 days. Blood glucose levels subsequently decreased from 250 mg to 141 mg/dL in the experimental group. The same research team investigated the effects of A. vera gel in combination with a standard oral antidiabetic therapy (2 × 5 mg oral glibenclamide) and the subjects received either Aloe or placebo as above. Results showed similar decreases in blood glucose in the actively treated group as described in the first trial. However, these studies were neither randomized nor blinded to patient or investigator. **[35]** designed a double-blind, placebo-controlled pilot study of two Aloe products (UP780 and AC952) in patients with prediabetes over an 8-week period. A group of 45 subjects with impaired fasting glucose or impaired glucose tolerance was recruited. Parameters such as fasting glucose, insulin, homeostasis model assessment (HOMA), HbA1c, fructosamine, oral glucose tolerance test, and oxidative stress (urinary F2-isoprostanes) were measured along with lipid profile and high-sensitivity C-reactive protein levels before and after supplementation. Compared to the placebo, only the AC952 A. vera inner leaf gel powder resulted in a significant reduction in glucose and fructosamine. In the UP780 A. vera inner leaf gel powder standardized with 2% aloesin group, there were significant reductions in HbA1c, fructosamine, fasting glucose, insulin, and HOMA. Only the UP780 aloe group had a significant reduction in F2-isoprostanes compared to placebo group. After evaluation of these results, the authors considered that standardized aloe preparations offer an attractive adjunctive strategy to revert impaired fasting glucose and impaired glucose tolerance observed in conditions of prediabetes/metabolic syndrome.

It is known as Ghikanvar which belongs to Liliaceae family. It looks like a cactus plant with green blade shaped leaves that are heavy narrowing, hairy and filled with clear viscid gel. Oral administration of aqueous extract of aloe Vera in a dose of 150mg/kg of body weight significantly lowering the blood glucose level. Aloe Vera gel consist various therapeutic effects such as anti-diabetic, antioxidant, increases the decrease level of glutathione by four times in diabetic rats **[36].**

Neem (azadirachta indica)

It is locally name as neem which belongs to family Meliaceae. It is available in India and Burma **[37].** Ethanolic and aqueous extract of *Azadirachita indica* shows reduction in blood glucose level in high dose. It can be combined with allopathic drugs in type 2 diabetic patients whose diabetes is not maintained by allopathic drugs only. Worldwide large numbers of patients are treated by natural neem tablets. Its extract improves the blood circulation by enlarging the blood vessels and useful in reducing the blood glucose level in the body [38].

Cocoa,

Theobroma cacao L. (Sterculiaceae), and its derivatives are of interest in the prevention of cardiovascular disease **[39].** In a clinical trial, **[40]** studied the effect of flavanols from chocolate on different cardiovascular risk factors in hypertensive patients, including insulin sensitivity and β cell function. They used two groups of hypertensive patients, randomized to receive either flavanol-rich dark chocolate or flavanol-free white chocolate (100 g/day for 15 days). The results showed that the first group but not the second decreased insulin resistance and increased insulin sensitivity and β cell function. Some years later, **[41]** realized a systematic review on the effects of cocoa, chocolate, and flavan-3-ols on the classic risk factors, and also considered the reciprocal relation between insulin resistance and endothelial dysfunction as well as other independent predictors such as fasting glucose, insulin, and HbA1c. The review, including 42 acute or short-term, randomized controlled trials with 1297 patients, showed that chocolate or cocoa induced a significant reduction in serum insulin without negative effects, thus suggesting insulin resistance improvement **[42].**

Plate 4. posted organic hydrate lime for poly herbal (Mixture) in readiness for basification

Bitter melon

M. charantia is a climbing perennial plant that produces elongated fruits with a pronounced bitter taste, which is known as bitter melon or bitter gourd **[43].** This species has been studied in vitro and in vivo for its potential antidiabetic properties, with different parts of this plant (seeds, fruit pulp, leaves, and whole plant) and different doses (from 400 mg to 6 g/day) being as sayed. In experiments using rats, M. charantia improves tolerance, suppresses postprandial hyperglycemia, and enhances insulin sensitivity. Several mechanisms of action have been proposed such as induction of glucose uptake and increased adiponectin secretion **[44],** activation of the AMPK system, and PPARα and PPARγ receptor activation. PPARα and PPARγ are pivotal in lipid and glucose hemostasis and may mitigate insulin resistance [106]. Different compounds have been isolated and some of them have been implicated as potential active principles such as α eleostearic acid (9-cis, 11-trans, 13-trans octadecatrienoic acid) as a PPARα activator **[45].** However, a Zn-free protein bearing in sulinomimetic activities has been isolated from the fruit of this plant, which could be related to the protein called "vegetable insulin" reported 30 years ago. Nahas and Moher **[46]** reviewed previous clinical trials and cited one with 40 and a second with 51 patients in which no effects on HbA1c or fasting blood glucose were reported **[47]** compiled ten clinical studies performed until 2008, but only five were relevant and only four were designed as clinical trials. Some of these reported significant results in the reduction of both fasting and postprandial sugar

levels. However, the different size of the trials, forms of administration (methanol extract, dried powder, fresh fruit or enriched fraction), dosage (timing and dose), and outcome measures (HbA1c, postprandial sugar levels or oral glucose tolerance test), use of the same subjects as controls and trials, different reports on adverse effects, etc., render these studies only relatively interesting for the use of this medicinal plant as an antidiabetic

Coffee

Seeds of coffee, Coffea arabica L. (Rubiaceae), intake is associated with a reduced risk of T2DM. To confirm this property, **[48]** examined the long-term relationship between the consumption of coffee and other caffeinated beverages and the incidence of T2DM in a prospective cohort study (The Nurses' Health Study and Health Professionals Follow-up Study). They followed up 41 934 men (1986 to 1998) and 84 276 women (1980 to 1998) without diabetes, and 1333 new cases of T2DM in men and 4085 new cases in women were documented. They found an inverse association between coffee intake and T2DM, thus suggesting that long-term coffee consumption is associated with a significantly lower risk for the disease **[49].** This effect was associated with mineral and antioxidant contents, but the role of caffeine was not specified until the Pereira et al. study **[50].** These authors demonstrated in a prospective analysis with a cohort of 28 812 postmenopausal women free of diabetes of the Iowa Women's Health Study (1986–1997) that coffee intake, especially decaffeinated, was inversely associated with a risk of T2DM.

Garlic (allium sativum)

Allium sativum L. (Alliaceae), has been used in India for its antidiabetic properties since ancient times **[51].** In recent years, different in vitro and in vivo studies demonstrated garlics antihyperglycemic effects. Ackermann et al. **[51]** reviewed the effects of garlic on several cardiovascular-related factors and its adverse effects; after analyzing 45 randomized trials, they observed no effects on glycemic-related outcomes. In their conclusions, the authors recommend future studies with clear definitions of constituents and preparations, because in these clinical trials there are great variations of samples (oil macerate, aged garlic, and different kinds of extracts) and doses (from 10 mg to 10 g). Some years later, Mohammadi and Oshaghi **[52],** working with mice, observed that garlic extract antagonized LXRα, an important regulator of cholesterol, triglycerides, and glucose homeostasis, and increased LXRα expression in the intestine. These effects could have an important role in the reduction of the lipid profile by garlic, which would justify the potential for this treatment of diabetes, but it should be demonstrated in humans.

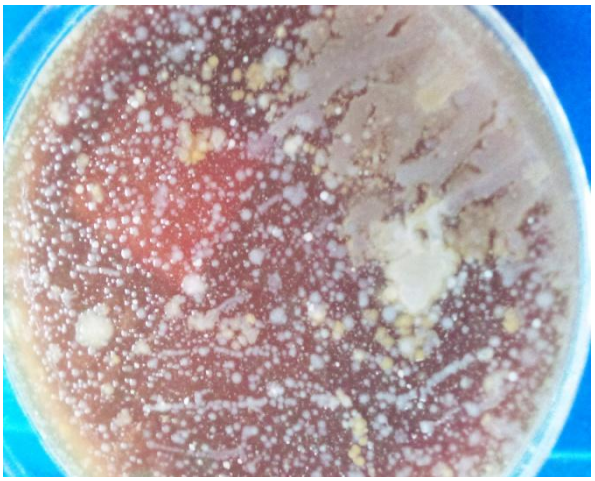

Plate 5. posted some motile mixed culture of bacteria for fermented drug discovery

Guava

Psidium guajava L. (Myrtaceae) or guava, also known as guayaba (Spanish), is a food crop and medicinal plant from tropical countries whose leaves (water extract) are used to reduce hyperglycemia in diabetic patients in Mexico. Many papers describing its pharmacological activities have been published, and Gutiérrez et al. reported two clinical assays **[53].** In one paper in China, a multicentric, randomized, controlled trial evaluated the efficacy of guava in diabetes management. After oral administration of 500 mg of aqueous leaf extract to 50 diabetic patients, they considered that guava could be used as a complementary therapy for preventing and treating diabetes mellitus, but not as a principal agent, since it is less effective than standard drugs. In the second trial, oral administration of 500 mg (fruit) to 40 patients decreased glycemia after 3 weeks of treatment compared to the diabetic control group

2.8 OXIDATIVE STRESS, DIABETES, AND ANTIOXIDANTS

The metabolic abnormalities of diabetes cause mitochondrial superoxide over-production in several tissues including the endocrine pancreas, which in turn activates pathways involved in the pathogenesis of complications and a further increase in intracellular reactive oxygen species (ROS) **[54].** Therefore, ROS seem to be causal agents in the pathogenesis of diabetes by damaging β cells. However, the removal of too many ROS may itself lead to metabolic dysfunction and predisposition to diabetes **[5**5]. Consequently, since oxidative stress plays a key role in insulin resistance and β cell dysfunction, administration of antioxidants could help to reduce diabetic complications, but it should be considered that a drastic reduction of radicals such as NO after an antioxidant therapy could be implicated in future cardiovascular diseases associated with diabetes. Therefore, different studies were oriented towards this goal. Some examples of species with positive effects are compiled in **[55]. [57]** evaluated the effects of curcumin and insulin on antioxidant enzyme activity in blood, liver, and kidney as well as on lipid peroxidation in rats. Histopathological analysis showed that treatment with insulin improved renal and hepatic lesions from both the diabetic insulin-treated group and the diabetic insulin/curcumin-treated group, as well as thiobarbituric acid reactive substance levels in serum, liver, and kidney of the treated groups. Rats treated with curcumin and insulin presented an increase in catalase activity, revealing a positive interaction between both substances. This treatment also prevented the oxidative stress in blood, which was reduced through the modulation of enzymatic antioxidant defenses **[58].** Otostegia persica Boiss (Lamiaceae) significantly decreased glycemia in diabetic rats 1–4 h after treatment, parallel to an increase in the serum insulin level. This extract also significantly decreased MDA and increased GSH levels in the liver of diabetic rats. The authors identified thymol as the major compound in the active extract **[59].** Cyanidin-3-O-β-D-glucopyranoside from mulberry (Morus alba L., Moraceae) fruits has protective effects against oxidative damage in streptozotocin-induced diabetic rat bladder **[60].** Achyranthes aspera Duss (Amaranthaceae) ethanolic extracts showed antioxidant activity and significantly reduced blood glucose levels in alloxan-induced diabetic mice. This extract also prevented lipid peroxidation and hydroperoxides, increased catalase activity, and reduced nitric oxide levels in the same experiments. The authors conclude that antihyperglycemic activity of A. aspera extracts could be mediated by oxidative stress reduction **[61].** The methanolic extract of Picralima nitida Th. & H.Dur. (Apocynaceae) and the hydroethanolic extract of Sonchus oleraceus Wall. (Asteraceae) showed significant antidiabetic activities, with a 39% reduction in glycemia, a significant reduction in MDA and hydrogen peroxide levels, and a substantial increase in catalase activity **[62].** Murraya koenigii Spreng. (Rutaceae) and Olea europaea L. (Oleaceae) reduced serum glucose levels by 56% and 67%, respectively, compared to the metformin group (63%). The authors hypothesized that antioxidants such as carbazole alkaloids and polyphenols in the extract could be responsible for this activity [101]**[63].** Berberine is an alkaloid found in different medicinal plants such as Berberis vulgaris L. (Berberidaceae), Coptis chinensis Franch. (Ranunculaceae), and Hydrastis canadensis. Poir. (Ranunculaceae). It showed antioxidant activity due to its scavenger properties against superoxide free radicals, an increase of sirtuin 1 expression, and attenuation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression. Activation of NADPH oxidase is associated with diabetes, and is now considered a potential target to treat the illness and related complications. Therefore, inhibition of NADPH oxidase could partially explain the beneficial effects of berberine on diabetic complications. Inflammation is also critical for the pathogenesis of T2DM, and berberine has shown anti-inflammatory properties, probably due to its capacity to inhibit mediators and transcription factors such as TNF-α, IL-6, IL-1β, matrix metalloproteinase-9, cyclo-oxygenase-2, iNOS, AMPK, MAPK, nuclear factor erythroid 2-related factor 2 pathway, and NF-κB pathway, which reduced the inflammatory response in T2DM. In conclusion, berberine antioxidant and anti-inflammatory activities could contribute to its therapeutic efficacy against T2DM and insulin resistance **[63].** Apocynin and lipoic acid are compounds with high potential as antidiabetic agents. Both compounds are widely distributed in the plant kingdom, and could be the active principles in studied or not yet investigated medicinal plants. Lipoic acid is common in plants of the Brassicaceae family (broccoli and watercress) but also in spinach and potatoes, whereas apocynin is common in Picrorhiza kurroa Royle ex Benth. (Scrophulariaceae) but also in other medicinal plants such as Jatropha multifida L. (Euphorbiaceae) and Apocynum cannabinum L. (Apocynaceae). **[64]** demonstrated that lipoic acid prevented hyperinsulinemia, hypertriglyceridemia, and insulin resistance, and improved hepatic insulin sensitivity and glucose tolerance. In the case of apocynin, these authors **[65]** also demonstrated the role of NADPH oxidase in fructose-rich diet-induced hepatic oxidative

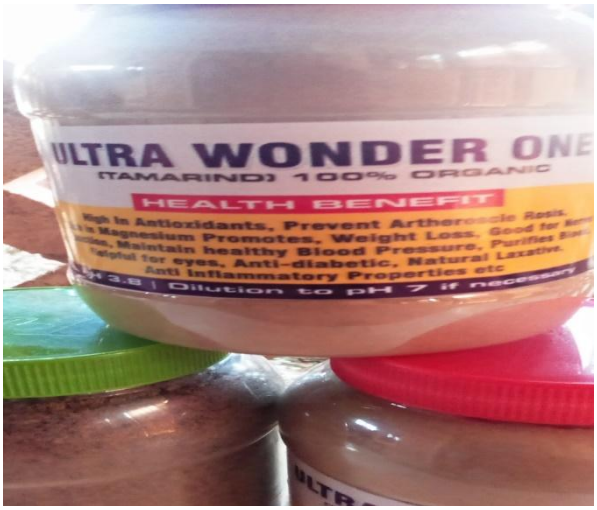

Plate 6. Posts processed tamarind which contain tartaric acid for acidification(buffer) of polyherbal mixture phase I

2.9 THE ROLE OF VITAMINS AND MINERALS ELEMENT IN MANAGEMENT OF DIABETES MILETUS

Vitamins Because diabetes is a state of increased oxidative stress, antioxidant vitamins are prescribed to people with diabetes **[66].** The following is a discussion of selected vitamins associated with lowering of blood glucose levels in type 2 diabetes patients. Vitamin E: The purported effects of vitamin E on glucose control relate to the vitamin's potent lipophilic antioxidant activity, with possible influences on protein glycation, lipid oxidation, and insulin sensitivity and secretion. Through unknown mechanisms, it also affects nonoxidative glucose metabolism **[67].**

A -lipoic acid:

α-lipoic acid is a potent lipophilic antioxidant. It is a cofactor in many multienzyme complexes (for instance, Pyruvate dehydrogenase multienzyme complex) and may also play a role in glucose oxidation12. In vitro α-lipoic acid enhances glucose uptake in muscle and prevents glucose-induced protein modifications **[67].**

Vitamins B6:

Vitamin B6 supplementation offers significant protection against the development of diabetic neuropathy as diabetes patients with neuropathy are deficient in vitamin B6 and benefit from its supplementation. Individuals with long-standing diabetes or who are developing signs of peripheral nerve abnormalities are supplemented with vitamin B6. The neuropathy of a vitamin B6 deficiency is indistinguishable from diabetic neuropathy. Vitamin B6 is also important in preventing other diabetic complications because it inhibits glycosylation of proteins **[68].**

Vitamin c:

Vitamin C reduces glycosylation and provides antioxidant activity, which is beneficial to diabetics **[69].**

Vitamin b12:

A vitamin B12 deficiency is characterized by numbness of the feet, pins and needles sensations, or a burning feeling (symptoms typical of diabetic neuropathy) [70]. Vitamin B12 supplementation has been used with some success in treating diabetic neuropathy. It is not clear if this is due to the correcting of a deficiency state or the normalization of the deranged vitamin B12 metabolism seen in diabetes patients. **BIOTIN:**

Biotin improves glucose metabolism and nerve function. Biotin supplementation enhances insulin sensitivity and increases the activity of the enzyme glucokinase, the enzyme responsible for the first step in the utilization of glucose by the liver. Glucokinase concentrations in diabetes patients are very low. In one study, a dose of 16 mg/day of biotin resulted in significant lowering of fasting blood sugar levels and improvements in blood glucose control in type I diabetes patients **[71].**

Minerals Deficiencies of certain minerals, such as potassium, magnesium, zinc and chromium aggravate carbohydrate intolerance **[72].** The following minerals are discussed in relation to their potential to manage diabetes mellitus;

Vanadium:

Prior to the discovery of insulin in 1922, vanadium was used for the control of blood sugar. Two small studies (one with six type 2 diabetes patients, and the next one with seven type 2 diabetic patients) showed that Vanadyl sulfate at a dose of 100 mg/day improves insulin sensitivity**[73].** Vanadium deficiency in human has not been documented. There are no accurate assays in clinical settings, and there is no recommended daily allowance. Vanadium exists in several valency forms, with vanadyl (+5) sulfate and sodium metavanadate (+4) being the

most common supplement forms. Its mechanism of action in glycemic control is thought to be primarily insulin-mimetic with up regulation of insulin receptors. In animal models, it facilitates glucose uptake and metabolism and enhances insulin sensitivity. Clinically, it enhances glucose oxidation and glycogen synthesis, and it modulates hepatic glucose output. Gastrointestinal discomfort, including diarrhea, nausea, and flatulence, are the side effects of administration of vanadium salts to patients. Organically chelated compounds, however, are thought to cause less gastrointestinal irritation than vanadium salts **[74]**

Plate7. Posts processed tamarind which contain tartaric acid for acidification(buffer) of polyherbal mixture phase II

Chromium:
Chromium (Cr3+), a trace element in its trivalent form, is required for the maintenance of normal glucose metabolism. Experimentally, chromium deficiency is associated with impaired glucose tolerance, which is improved with supplementation **[75].** Most individuals with diabetes, however, are not chromium deficient. It is a part of glucose tolerance factor (GTF), a biologically active substance manufactured in the body that regulates glucose biotransformation and increases the number of insulin receptors, enhances receptor binding, and potentiates insulin action. Chromium picolinate is the preferred form because it is utilized more efficiently **[76].** Chromium administration decreases fasting and postprandial glucose and decreases fatigue, excessive thirst, and frequent urination25. No recommended daily allowance (RDA) exists for chromium. A good supply of chromium is assured by supplemental chromium26 in addition to dietary sources. Good dietary sources are brewer's yeast27 and barley flour28, while refined sugars, white flour products, and lack of exercise deplete chromium levels **[77].**

Magnesium:
A deficiency of magnesium is significantly more common in type 2 diabetics than in the general population especially those with glycosuria, ketoacidosis, and excess urinary magnesium losses **[78]** Magnesium deficiency is associated with complications of diabetes, retinopathy in particular. One study observed that the patients with the most severe retinopathy had also the lowest levels of magnesium30. Deficiency of magnesium potentially causes states of insulin resistance. Magnesium is a cofactor in various enzyme pathways involved in glucose oxidation, and it modulates glucose transport across cell membranes. It increases insulin secretion and/or improves insulin sensitivity and peripheral glucose uptake. It has no effect on hepatic glucose output and non-oxidative glucose disposal. Because it is an intracellular cation, it is difficult to measure accurately, and total body stores are rarely measured **[79].**

Calcium:
A daily intake of 1,000–1,500 mg of calcium, especially in older subjects with diabetes, is recommended. This recommendation is safe and reduces osteoporosis in older persons. Piero et al. value of calcium supplementation in younger persons is uncertain9. Calcium improves insulin sensitivity in some type 2 diabetic populations **[80].**

Manganese:
Manganese is essential for human health. It is a cofactor of various enzymes that aid in cellular biochemical reactions. Such reactions include making and activating manganese superoxide dismutase (MnSOD) (an antioxidant enzyme) that helps protect the cell membranes and tissues from degeneration and disruption, helping the body to catabolize carbohydrates, lipids and proteins, and assisting in energy production32. Manganese

deficiency causes impaired glucose tolerance, impaired growth, impaired reproductive function, skeletal abnormalities, and altered carbohydrate and lipid metabolism **[81]**.

Potassium:

Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer **[82].**

Zinc:

Zinc is involved in virtually all aspects of insulin metabolism: synthesis, secretion and utilization. Zinc also has a protective effect against β-cell destruction and has anti-viral effects. Diabetics typically excrete excessive amounts of zinc in the urine and therefore require supplementation. This improves insulin levels in both type 1 and type 2 diabetes. In addition, zinc helps improve the poor wound healing observed in diabetes patients. Zinc is found in good amounts in whole grains, legumes, nuts, and seeds. The recommended level of supplementation for diabetics is at least 30 mg of zinc per day **[83].**

Selenium:

Selenium is an important component of seleno-proteins, which are implicated in modulating oxidative stress and regulating thyroid hormone activity **[84].** Two recent studies undertaken to examine the relationship between serum selenium levels and the prevalence of diabetes among U.S. adults established that high serum selenium levels were positively associated with the prevalence of diabetes40, that selenium supplementation did not prevent type 2 diabetes, and that it may increase the risk for the disease41. Therefore, the in-discriminant use of selenium supplements should be discouraged until more randomized, controlled trials examine their effects on human health.

III. MATERIALS AND METHOD

3.1 INSTRUMENTS

Steam pressure cooker (Autoclave), Weighing machine (rove Lectronics) 20µl-micropipette, light microscope (Carzeiss), Hot air oven (kasliwal brothers), Refrigerators (whirlpool GNF 220)

Glass wares, Plastic wares and consumable

Conical flasks, Beaker, Petri plates, Stirrer, Pipette, Test tube stand, culture tubes, slides, Reagent bottle, cover slips, Forceps Scalpels, Scissors, Wash bottle, Sucker, Gloves, Funnel, Burner tips, Tissue paper, Aluminum foil, filter paper, Test tube, cotton etc., respectively

3.2 MEDIA USED

1. Nutrient Agar media(conventional)
2. Blood chocolate media (home Base)
3. Media for isolation of *Bacillus* sp, *Klebsiella* sp, *Pseudomonas* sp etc. (Home base)

3.3 MICROOGANISM ISOLATION AND IDENTIFICATION

Isolation of bacteria from a mixture of processed cholate blood dry agar medium/ Soya been milk when expose in an ordinary room for three days. samples Serial weakening methods were utilized for the confinement of microscopic organisms. In this procedure test suspension was set up by including the processed blood agar / soya bean milk after blending (1g) was added to 10 ml of sterile water (the stock) and shaken overwhelmingly for no less than 1 minute. The weaken was then sedimented for a brief period. Sterile weakening spaces were stamped successively beginning from stock and 10^{-1} to 10^{-4}. One ml from the stock was exchanged to the 10-1 weakening clear utilizing a crisp sterile pipette. One ml from the 10-1 weakening was exchanged to the 10^{-2} tube for each succeeding stride then from the 10^{-2} to the 10^{-3}, then from the 10^{-3} to the 10^{-4}. From every weakening tube 0.1 ml of weakening liquid was moved into Nutrient Agar society media and hatched at 37 °C for 24 hours **[85].** Ingredient used for the processed of home base medium were as follows: meat extract- 500ml, salt-10g, sugar cane molasses- 20g, soya bean milk and processed blood extract, hydrated organic base KOH was use for home base pH adjustment to pH 7 and above. Supplements for conventional media Nutrient agar (NA) society media contained 0.5% peptone, 0.3% yeast separate, 0.5% NaCl, 0.25% glucose, 1.5% agar, refined water and PH was acclimated to 7 at room temperature. After fruitful development of microorganisms as **seen in plate 1, 5, 6, 7 and 8** the unadulterated societies of microscopic organisms were sub-refined in NA inclines; hatched at 37 °C to accomplish enthusiastic development and afterward safeguarded in 20% glycerol vials at - 80 °C

Plate 8. posts mixed culture *Bacillus* sp and *Enterobacter* sp

3.4 MICROBIOLOGICAL AND BIOCHEMICAL CHARACTERISTIC OF ISOLATED BACTERIA

Gram stain was performed to watch the cell morphology and gram nature of the microorganisms and biochemical portrayal of the strains were additionally completed. The biochemical tests of Sugar usage; Amino corrosive decarboxylation; Catalase and oxidase creation; Nitrate lessening; Hydrogen sulfide generation; Starch, Casein and Urea hydrolysis; IMVIC tests were performed in-situ [86].

3.5 SELECTION OF OPTIMAL GROWTH MEDIUM FOR THE SYNTHESIS ALKALINE PROTEINASE FROM BACILLUS SP PSEUDOMONAS SP KLEBSIELLA SP TO MILD ACIDIC THROUGH BUFFERING USING HOME BASE MATERIALS (INDIGENOUS)

Comparative study for fermentation media and culture conditions

To study the biosynthesis of protein from *Bacillus* sp *Pseudomonas* sp and *Klebsiella* sp two different growth media were used the conventional and home base for alkaline proteinase production were used. The control Medium was used as control while the home base was use as the treated medium. The conventional medium had the following composition (g/l): glucose,2.0; Casein, 0.5; peptone, 0.5 yeast extract, 0.5 salt(v/v), 5%, (mgSO4. 7H2O 5.0, KH2PHO4 5.0, FeSO4. 7H2O 0.1) **[87].** Home Base Medium (sugar cane molasses, 20g; process soya bean milk 50ml, chocolate blood past 50 ml; Sodium Chloride 20g; processed yeast extract 30ml; meat extract 50ml; processed peptone, as well as all the plants extract/seed contain an appreciable amount of P, K, Mg, Mn, Fe/ Vitamins). Basal medium containing the synthetic peptone as well as home base peptone alongside with both synthetic yeast extract and home base (each at 1.5 g/l level. After autoclaving and cooling the pH of all the media were adjusted to 7.0 and above by addition of sodium bicarbonate for synthetic basal medium while Hydrated process Organic KOH/NaOH for the home base basal medium. Fermentation experiments were set to be carried in 50ml of the above described medium in 250 ml Erlenmeyer flasks. 5% of an 18 h old inoculum raised in the basal medium was used to initiate growth. The inoculated flasks were kept on an environmental shaker (New Brunswisk) at 120 rpm for 12 days at 30^{O}C. Samples were taken for every 24h and was estimated for enzyme activity, soluble protein and growth. All the experiments were carried out in duplicate.

Plate 9. posted final heat boiling of the mixture of the fermenter containing polyherbal mixture (antioxidant) as well as checkmating the pH to mild acidity (under processed tartaric acid powder)

3.6 ANTIMICROBIAL ASSAY OF THE CRUDE EXTRACTS:

The antimicrobial activity was measured by the agar well diffusion method **[88].** Sterile nutrient agar was prepared and placed in labelled Petri dishes and allowed to gel. The antibacterial culture was prepared using the test organism on (**Nutrient agar/home base nutrient agar**). Four wells were bored into the nutrient agar using a 7 mm sterile corked borer and inoculums containing 10^5 CFU/ml of bacteria spread on the solid media with sterile cotton swab moistened with bacterial suspension. Fifty microliters (50 mg/ml) of essential oils and fractions were dispensed in each well. The dried solvent extracts (0.2 g) were reconstituted in 2 ml of normal saline and distilled water respectively, to make a stock solution with a 24 concentration of 100 mg/ml. as shown **in table 6. plates** 11, 12, 13 &14

3.7 SELECTION OF PLANTS AND EXTRACT PREPARATION FOR BIOACTIVITY EVALUATION

Plant material:

Eleven medicinal plants were employed for the diabetic type I and Type II cured were used as noted in the research and they are as followed. ***Beta vulgaris*** (beet root), ***Ziniber officinale***(zinger), Moringa leaf/seed (Moringa ***oleifera)***, (***Comellia sinensis***)green tea), Bitter cola(***Garcinia Kola***), (***Mentha***) Mint, Tomato(***Solanum lycopersicum***), Turmeric(***Curcuma longa***), (***Citrus aurantiifolia)*** lime juice, (***Tamarindus indica)*** tsamiya, and (***Saccharum Officinarum***) sugar cane. Thus, they were collected through purchasing in the market while some were arbitrarily collected from different locations in farm land, Riverine areas as well as bushes free of charge (Kaduna Metropolis) in Kaduna State of Nigeria.

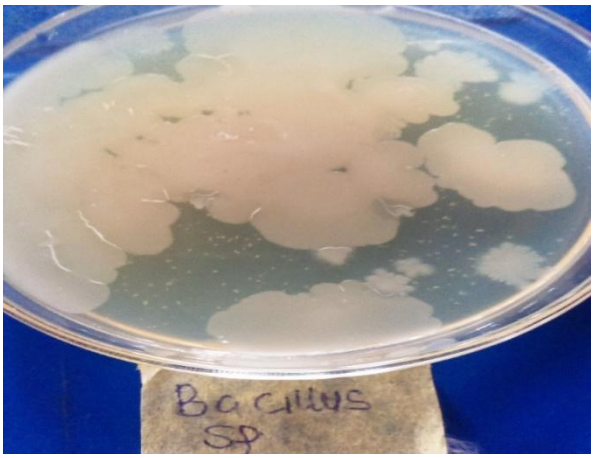

Plate 10. posted Bacillus sp grown on solid agar medium using solid home base agar medium

3.8 PREPARATION OF PLANT EXTRACTS;

Plant parts were collected and wash with water, then dry at room temperature and ground to a fine powder. Aqueous extracts (1:10 w/v) were left at room temperature (25-28^{O} C) for 24 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 10 min. The supernatant was collected. This procedure was repeated twice after which the filtrates were evaporated by a vacuum rotary evaporator to make crude extracts and store at 4°C until used.

3.9 THE USE OF WATER, ETHANOL, METHANOL, ACETONE

In phytochemistry Since herbal medicine research aims at identifying bioactive phytocompounds in medicinal plant extracts used by local people to treat diseases based on indigenous knowledge, the solvent chosen must be the same as that used by local communities. Hence, water and ethanol are the commonly used solvents. Water is a universal solvent used to extract plant products with antimicrobial activity and ethanol (at a concentration of 70-96%) is more effective in isolating bioactive phytocompounds than water. While other solvents were momentarily used so as to ascertain whether they could be tenaciously used for extraction of bioactive compounds as well. Since this study aims at validating indigenous knowledge for diabatic type I and type II, water and ethanol extracts were prepared for ethnopharmacological screening. Nearly all identified components from plants active against microorganisms are aromatic and saturated organic compounds, and are most often obtained through ethanol and water extraction **[89].**

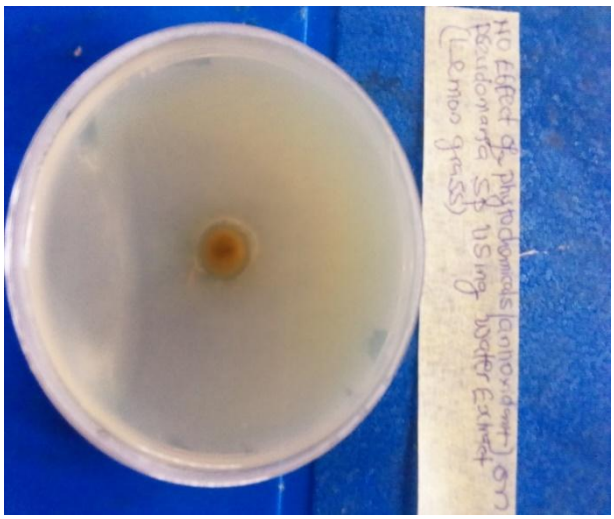

Plate 11. posted the effect of lemon grass water extracts on Bacillus sp using methanol solvent

3.10 PHYTOCHEMICAL SCREENING:

Major phytoconstituents in the test plant extract such as alkaloids, saponins, Tannin, steroids, flavonoids, glycosides, terpenoids anthraquinone etc., qualitative phytochemical analysis The bioactive constituents present in different solvent extracts was qualitatively analyzed following standard procedures as described by Harborne, Treasa and Evans1 and Softwara **[90]**

Test for Saponin

Methodology is as reported by **[91].** Distilled water (30 cm^3) was added to the plants extract samples (0.30 g) and boiled for 10 minutes in water bath and filtered using Whatman filter paper number 42 (125 mm). A mixture of distilled water (5 cm^3) and filtrate (10 cm^3) was agitated vigorously for a stable persistent froth. The formation of emulsion on addition of three drops of olive oil showed positive result.

Test for Steroid

Analytical method used is according to **[92].** Each sample (0.30 g) weighed into a beaker was mixed with 20 cm^3 of ethanol; the component was extracted for 2 hours. To the ethanolic extract of each sample (5 cm^3) was added 2 cm^3 acetic anhydride followed with 2 cm^3 of concentrated tetra-oxosulphate (VI) acid. A violet to blue or green color change in sample(s) indicates the presence of steroids.

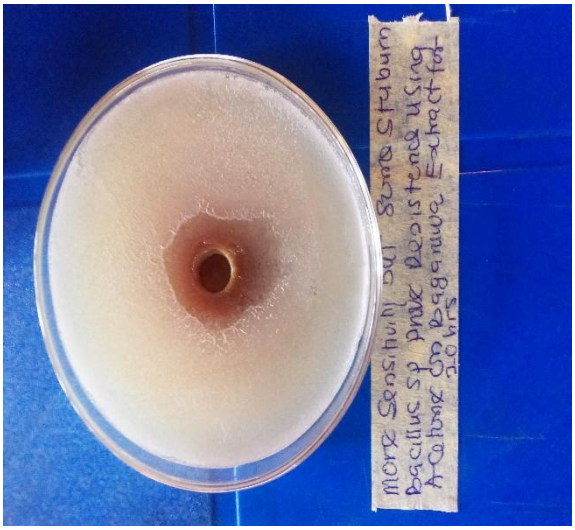

Plate 12. Posts the susceptibility of bacillus sp on Bagaruwa extract using Acetone solvent

Test for Terpenoids

Methodology is as reported by **[93].** Each of the plant extract sample (5ml) was introduce into a beaker and extracted with 30 cm^3 and component extracted for 2 hours. A mixture of chloroform (2 cm^3) and concentrated tetra-oxosulphate (VI) acid (3 cm^3) was added to 5 cm^3 of each extract to form a layer. The presence of a reddish-brown coloration at the interface shows positive results for the presence of terpenoids.

Test for Flavonoids

The test for flavonoid adopted is as reported by [94]. Each sample (0.30 g) weighed into a beaker was extracted with 30 cm^3 of distilled water for 2 hours and filtered with Whatman filter paper number 42 (125 mm). To 10 cm^3 of the aqueous filtrate of each wood extract was added 5 cm^3 of 1.0 M dilute ammonia solution followed by the addition of 5 cm^3 of concentrated tetra-oxosulphate (VI) acid. Appearance of yellow coloration which disappeared on standing shows the presence of flavonoids.

Test for Alkaloids

Test for alkaloids: A total of 0.5 g each of the shoot and root powder of plants was mixed with methanol containing 1% HCl, and then boiled and filtered. A total of 2 ml of 10% ammonia and 5 ml of chloroform was added to 5 ml of the filtrates and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 2 ml of acetic acid, and Mayer's reagent was added. The formation of cream (with Mayer's reagent) or presence of turbidity was regarded as the presence of alkaloids**. [95]**

Test for Glycoside

Glycoside test was conducted according to the method reported by **[96].** To 2.00 g of each sample was added 20 cm^3 of water, heated for 5 minutes on a water bath and filtered through Gem filter paper (12.5 cm). The following tests were carried out with the filtrate:(a)0.2 cm^3 of Fehling's solutions A and B was mixed with 5 cm^3 of the filtrate until it became alkaline (tested with litmus paper). A brick-red coloration on heating showed a positive result. (b)Instead of water, 15 cm^3 of 1.0 M sulphuric acid was used to repeat the above test and the quantity of precipitate obtained compared with that of (a) above. High precipitate content indicates the presence of glycoside while low content shows the absence of glycoside.

Test for anthraquinone

About 0.5 g of the shoot extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with an equal volume of 10% ammonia solution. A pink violet or red color in the ammonia layer was observed which indicative the presence of anthraquinones **[97].**

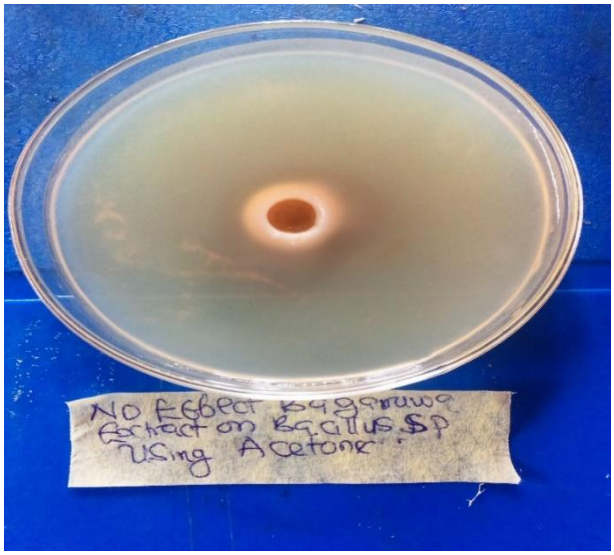

Plate 13. Posted Bacillus sp growth on Bagaruwa extract using acetone (resistant)

Test for Tannins:

Water extracts of plant shoots were treated with 15% ferric chloride test solution. A blue color in the mixtures signified the presence of hydrolysable tannin. For confirmation, 0.5 g of the extracts were added to 10 mL of freshly prepared potassium hydroxide (KOH) in a beaker, and shaken to dissolve. A dirty precipitate was indicative the presence of tannins **[98]**

3.11 QUANTITATIVE ESTIMATION OF SECONDARY METABOLITES

The presence of secondary metabolites from the leaves, roots and stem bark of the test plants were quantitatively determined by adopting standard protocols

Determination of alkaloids

Quantitative determination of alkaloid was according to the methodology by **[99].** Exactly 200 cm^3 of 10% acetic acid in ethanol was added to each wood powder sample (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). Using electronic weighing balance Model B-218, the residue was dried in an oven and the percentage of alkaloid is expressed mathematically as

$$\%\text{Alkaloid} = \frac{\text{Weight of Alkaloids} \times 100}{\text{Weight of the sample}}$$

Determination of flavonoid

Flavonoid determination was by the method reported by **[100].** Exactly 50 cm^3 of 80% aqueous methanol added was added to 2.50 g of sample in a 250 cm^3 beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was reextracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each wood sample. Each wood sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as

$$\%\ \text{Flavonoid} = \frac{\text{Weight of Alkaloids} \times 100}{\text{Weight of the sample}}$$

Determination of saponin

Saponin quantitative determination was carried out using the method reported by **[101].** Exactly 100 cm^3 of 20% aqueous ethanol was added to 5 grams of each wood powder sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was reextracted with another 100 cm^3 of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90°C. 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage:

$$\%\text{Flavonoid} = \frac{\text{Weight of Alkaloids X 100}}{\text{Weight of the sample}}$$

Determination of tannins

Analytical method for quantitative determination of tannin was according to **[101].** By dissolving 50 g of sodium tungstate (Na_2WO_4) in 37 cm^3 of distilled water, Folin-Denis's reagent was made. To the reagent prepared above, 10 g of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and 25 cm^3 of orthophosphoric acid (H_3PO_4) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 cm^3 with distilled water. One gram of each wood powder (sample) in a conical flask was added to 100 cm^3 of distilled water. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper in a 100 cm^3 volumetric flask. Addition of 5.0 cm^3 Folin-Denis reagent and 10 cm^3 of saturated Na_2CO_3 solution into 50 cm^3 of distilled water and 10 cm^3 of diluted extract (aliquot volume) was carried out after being pipetted into a 100 cm^3 conical flask for colour development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25°C after thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer optical density was measured at 700 nm and compared on a standard tannic acid curve. Dissolution of 0.20 g of tannic acid in distilled water and dilution to 200 cm^3 mark (1 mg/cm^3) were used to obtain tannic standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-Denis's reagent (5 cm^3) and saturated Na_2CO_3 (10 cm^3) solution were added and made up to the 100 cm^3 mark with distilled water. The solution was left to stand for 30 minutes in a water bath at 25°C. Optical density was ascertained at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. Optical density (absorbance) versus tannic acid concentration was plotted.

The following formula was used in the calculation:

$$\text{Tannic acid (mg)} = \frac{\text{C x Volume x 100}}{\text{Aliquot Volume x weight of sample}}$$

Plate 14 posted Sodium apple extract on the growth of Bacillus sp using water extract(resistivity)

Test for Terpenoids

Dried plant extract 10 gram (Wi) was taken and soaked in 90 ml of ethanol). The extract after filtration was mixed with 10 ml of petroleum ether and again filtrated using separating funnel. The extract was waited for its complete drying and measurement is taken (Wf). The yield (%) of total terpenoids contents was measured by the formula:

$$\text{Total terpenoids} = \frac{Wi - Wf}{\text{Wi}} \times 100$$

Where, Wi= dried plant extracts,

Wf= extracts after drying **[102]**

Glycoside:

Determination of Cardiac gylcosides: Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described **by [104]** .1g/50ml of the fine powder/ filtrate of Senecio biafrae leaves was soaked in 10ml of 70% alcohol for 2hrs. and then filtered. The extract obtained was then purified using lead acetate and Na2HPO4 solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid + 5ml 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

The following formular was used in the calculation

% Inhibition= (abs of the test control - Abs of the test solution X 100)
Abs of control

Total anthraquinone glycosides

Quantitative Analysis of Total Anthraquinone Glycosides12; the extract (1.00 g) was accurately weighed and distilled water (30 ml) was added. The mixture was mixed, weighed and refluxed on a water bath for 15 minutes. The flask was allowed to cool, weighed, adjusted to the original weight with water and the mixture was centrifuged at 4000 rpm for 10 minutes. Twenty milliliter of the supernatant liquid was transferred to a separatory funnel and acidified with 2 M hydrochloric acid. Fifteen milliliter of chloroform was added, the mixture was extracted and the chloroform layer was discarded. The extraction was done triplicate. The aqueous layer was separated and 0.10 g of sodium bicarbonate was added. The mixture was then shaken for 3 minutes and centrifuged at 4000 rpm for another 10 minutes. Ten milliliter of the supernatant liquid was transferred to a 100 ml flask. The solution of 10.5% w/v ferric chloride hexahydrate (20 ml) was added and mixed. The mixture was refluxed on a boiling water bath for 20 minutes. Concentrated hydrochloric acid (1 ml) was added and the mixture was heated for 20 minutes, with frequently shaking to dissolve the precipitate. The mixture was cooled, transferred to a separatory funnel and shaken with 25 ml diethyl ether. The partition was repeated until anthraquinones were exhaustively extracted, tested by the Borntrager's reaction. The diethyl ether extracts were combined and washed with 15 ml distilled water twice. The combined diethyl ether was then transferred to a 100 ml volumetric flask and adjusted to volume. Twenty-five milliliter of the solution was evaporated to dryness. The residue was dissolved with 10 ml of 0.5% w/v magnesium acetate in methanol yielding a red solution. The UV absorbance was measured at 515 nm**.[105]**

The following formular was used in the calculation

% Inhibition= (abs of the test control - Abs of the test solution X 100)
Abs of control

3.12 DETERMINATION OF FERMENTED PROPERTIES OF SECONDARY METABOLITES

Theory.

pH is defined as the negative logarithm to the base10 of H^+ ion activity or concentration

pH= -log10 H^+ = log10 $1/H^+$(Activity) fermented secondary metabolite's reaction or pH is meant to express the acidity or alkalinity of the fermented microbial extract. It is very important property of the fermented secondary metabolites because it determines the capacity for the microbial growth absorbility of nutrient, bacterial activity and the physical condition of the fermented secondary metabolite growth

pH

Twenty-five ml of the fermented secondary metabolites was pipetted into 50 ml of a beaker, and 20 ml of reagent water was added after which it was then covered, and the suspended solution was continuously stirred for 5 minutes. This was then allowed to stand for about one hour so that the suspended clag or filter/centrifuged of the aqueous phase pH was measured. The electrode in the clamps of the electrode's holder was adjusted down into the beaker. The glass electrode was immersed just deep enough into the clear supernatant solution to establish a good electrical contact through the ground glass and was read on the pH meter **[106].**

Acidity

One hundred ml of the sample was introduced in an Erlenmeyer flask with three drops of phenolphthalein indicator. The solution was then titrated with 0.024 NaOH from a burette until the first permanent pink colour appeared and the 00 of sodium hydroxide used was recorded

Calculation

Millilitre of 0.02N NaOH x 10 = mg/L total acidity expressed as $CaC0_3$ [107]. Alkalinity

One hundred ml of the sample was introduced into a 250 00 Erlenmeyer flask followed by addition of three drops of phenolphthalein indicator to it. Upon the development of a pink color 0.02N sulphuric acid from a burette was added until the pink color just disappears and the amount of acid used (00) was recorded. Three drops of methyl orange indicator was then added to the flask. If the sample became yellow, then 0.02N Sulphuric acid was added until the difference in color was noted when compared with the distilled water. The alkalinity was calculated using:

Total alkalinity as $CaC0_3$ (mg/L) = Total ml acid x 10 **[108].**

Total Nitrogen

50 ml of the fermented sample was poured into 300 ml kjeldahl flask along with 25 ml of conc. H_2S0_4 and 3 g mixed catalyst. The sample was digested using Kjeldahl digestion apparatus until a clear green or whitish color was obtained. The digested solution was then diluted to 100 ml with distilled water. Distillation was done by adding 20 ml of diluted digest into 500 ml kjeldahl flask containing anti-bumping chips and 40 ml of 40% NaOH was slowly added by the side of the flask. A conical flask 250 ml) containing a mixture of 50 ml of 2% boric acid and 4 drops of mixed indicator (cresol/bromothymol) was used to trap the liberated ammonia. The distillate was then titrated with 0.IM Hel. The total nitrogen content was then calculated using

Calculation

$$\%N_2 = \frac{14 \times M \times Vt \times V}{\text{Weight of Sample (mg)} \times Vs} \times 100$$

Where M = actual molarity of acid

V = titre volume of HCI used

V t = total volume of diluted digest

Vs = aliquot volume distilled **[109].**

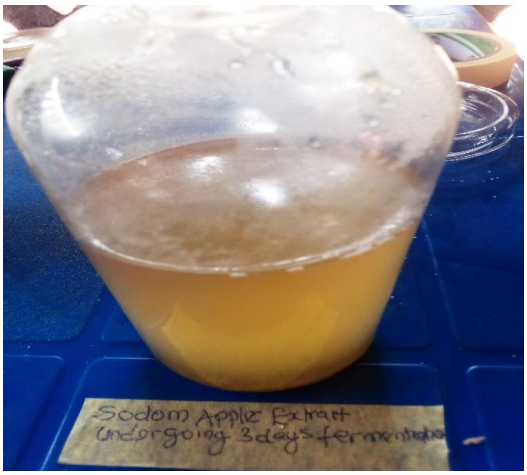

Plate 15. posted Sodom Apple extract (water extract undergoing fermentation

Phosphorus

Fifty ml of the fermented plant extract sample was filtered through a nylon cloth into a glass beaker. Twenty-five ml of the filtrate was heated for 25 minutes with HN03/HCI in a ratio of 3: 1 (digestion). The mixture was diluted to the 100 ml mark with distilled water. Fifteen ml of the diluted solution was then pipetted into a cuvette and I ml of the phosphate reagent was added to it and the mixture was shaken vigorously and the reading taken using the phosphate meter **[110].**

3.13. QUANTITATIVE ANALYSES OF MINERALS PRESENCE IN FERMENTED DIABATIC TYPE I AND TYPE II THERAPY

Potassium

To determine the potassium content of the mixed extract sample, 100ml of the mixture was introduced in 50 ml of distilled water and filtered using nylon cloth. The filtrate (25 ml) was mixed with HNO: $HCI0_4$ in a 2: I ratio. The beaker containing the mixture was then placed on a hot plate and boiled until the solution became clear. This was then filtered using Whatman filter paper No. I in a volumetric flask and the volume of the filtrate was made up to 100 ml by the addition of deionized water. Digested sample was stored in a sterile polyethylene bottle at room temperature for further analysis of the metal using atomic absorption Spectrophotometer
Obtained from NARICT,2006.

Calculation

% Potassium = *R* x 0.005
Where R = Potassium concentration (ppm) in the aliquot **[111]**

ZINC, CUPPER, MANGANASE AND IRON

THEORY

The chelating agents combine with free metal atom in solution to form complexes. DTPA (Diethlene trianminepenta acetic acid) offers the most favorable combination of stability constants for the simultaneous complexing of Zn, Cu, Mn, and Fe
Materials required

(i) DTPA 0.05M
(ii) CaCl2, 2H2O 0.01
(iii) TEA o.1 (Triethanol amine)

Extracting Solution. 13.1 ml reagent grade TEA, 1.967 g DTPA (AR Grade) and 0.5g of CaCl2 were Dissolved in 100ml of distilled water. The solution was allowed to settle for some time so as the DTPA could be allow to dissolved and dilute to approximately 900ml. The pH was adjusted to 7.3 with 1:1 HCL while stirring and dilute it to 1 liter.

Standard Solution. Zinc standard solution: 0. 439g AR grade ZnSO4. 7H2O was dissolved in 200ml distilled water in a beaker. 5ml of 1 NH2SO4 was as well added in the solution and was transferred to a liter- measuring flask. Thus, the volume was then marked by distilled water for a standard solution of 100ppm in-situ. 10 ml of this standard solution was dully Transferred to 100 ml volumetric flask which was then diluted to the mark with DTPA extracting solution for a stock solution of 100ppm. For the preparation of working standards solution and, transfer 1,2,4 and 6ml of stock solution (10ppm).
Volume of stock Zn solution taken: 0 1 2 4 6 ml
Conc. Of Zn now in solution: 0 0.1 0.2 0.4 0.6 (ppm)

Iron Standard Solution: 0.702 g of AR grade ammonium ferrous sulphate was poured in 300ml distilled water in a beaker after which 5 ml 1: 5 H2SO4 was added to the solution. the solution was later transferred to a flask of 1 liter capacity and make volume to the mark. This made a standard solution of 100 ppm in-situ. A working standard was prepared and transferred 1, 2, 4 and 6ml of stock solution and dilute each to the mark with DTPA extracting solution volume of stock Fe solution
Volume of stock Fe solution taken: 0 1 2 4 6 ml
Conc. Of Fe now in solution: 0 0.1 0.2 0.4 0.6 (ppm)
[112]

Manganese Standard Solution: 0.288g potassium permanganate was introduced in 300ml distilled water in a beaker after which 20ml conc. H2SO4 was then allowed to warm in water bath to about $60^{O}C$. Thus, oxalic acid solution was subsequently added drop wise to make the solution colorless. The Cooling solution was then transferred to a 1-liter flask and make volume to work. This made an equivalent of 100 ppm Mn solution. A working standard solution was circumspectly allowed by, transferring 1, 2, 4, 6 and 8 ml of the standard solution to series of 100ml volumetric flask and dilute each to the mark with DTPA extracting solution.
Volume of stock Mn solution taken: 0 1 2 4 6 8 ml

Conc. Of Mn now in solution: 0 0.1 0.2 0.4 0.6 8(ppm)

Copper Standard Solution:

0.392g copper sulphate in 400ml distilled water in a beaker was transfer to a little flask, and make volume to mark with distilled water. This standard solution containing 100 ppm Cu in-situ. A working standard solution was transfer 1, 2, 4 and 6 ml of stock solution to a series of clean 100ml volumetric flasks and dilute each to the mark with DTPA extracting solution.

Volume of stock Cu solution taken: 0 1 2 4 6 ml

Conc. Of Cu now in solution: 0 0.1 0.2 0.4 0.6 (ppm)

Procedure

At least 3-4 standards and a blank of each micronutrient cation are used for drawing a calibration curve. The blank solution 0 (ppm) is used to zero the atomic absorption spectrometer. The standards are then analyzed with the lowest concentration first, and the blank run between standards to ensure that the line (Zero point) has not changed. A graph of absorbance vs. Concentration of standard solution was plotted on a graph paper. After setting the instrument aspirate the sample, was read by absorbance, thus the concentration against absorbance from the curve was accentuated. 100ml of the fermented secondary metabolites extract was assiduously introduced into 250ml Erlenmeyer conical flask, after which 20 ml of DTPA extracting solution follows in-situ. The solution in the flask was tightly corked and shook for 2 hours with speed of 120cycles per minute. Further Filtrates in polypropylene bottle were then analyzed for the respective micronutrients in their sequence (Zn, Cu, Mn, Fe,) using atomic absorption spectrophotometer**. [113]**

3.14. QUALITATIVE ANALYSIS OF VITAMINS PRESENT IN FERMENTED DIABATIC TYPE I AND TYPE II THERAPY

Test for Vitamin – A In 5 ml of chloroform, 250mg of the powdered sample was dissolved and filtered in-situ. On to the filtrate, 5ml of antimony trichloride solution is added. The appearance of transient blue color indicates presence of vitamin- A **[114]**

A Test for vitamin – C In 5ml of distilled water, 1ml of the sample was diluted and a drop of 5% sodium nitroprusside and 2ml of NaOH was added. Few drops of HCl were likewise added dropwise, the yellow color turns blue. This indicates the presence of vitamin- C

Test for vitamin – D In 10 ml of chloroform, 50 mg /50ml of powdered/liquid extract was carefully dissolved and filtered as usual. 10ml of antimony trichloride was then added, the appearance of pinkish-red color indicates the presence of vitamin – D

Test for vitamin – E the extract of the sample was made and filtered (500mg in 10ml), few drops of 0.1% ferric chloride were added and 1ml of 0.25% of 2'- 2'dipyridyl was added to 1ml of the filtrate. Bright-red color was formed with a white background.

3.15. CELL BIOMASS DETERMINATION (DRY WEIGHT)

Following the methods used by **[115]** after completion of fermentation, the fermentation broth in each was filtered, the suspension in each flask were centrifuged at 5000 rpm for 10 minutes several times with intermittent washing with cold distilled water. Each biomass was dried in an oven at 60°C to constant weight. Dry weight of biomass cell was measured using Methler balance (Scaltee).

Plate 16. posted aseptically basification of a suitable medium from 7-8 for the growth of mutants' species in readiness for antidiabetic drugs discovery

IV. RESULTS AND DISCUSSIONS

Microorganisms are essential for the maintenance of sustainable ecosystem, microbial diversity, pharmacological and medicinal therapeutic treatment of patient. Thus, they are however often neglected due the inability for medical doctor, scientist, researcher etc., respectively who have a very few knowledge of exploration of God given natural endowment as well as the very good microorganism for pharmaceutical, medicinal and industrial usage. However, these microorganisms including bacteria, yeast and fungi occupy important niches in all ecosystem, pharmacology and medicinal which are responsible for recycling both microelement and microelement/ vitamins in nature(enzymatic). In addition, these microbes might also be que in for the decomposition, degradation of both organic matter and complex antioxidant compounds (toxic) to the barest minimum (harmless) amount judiciously, for human body good Healthwise care. Contrary wise, there have been detailed studies on the distribution of microbes in various environmental niches, medical as well as in pharmacological research program using phytochemical analysis so as to irradicate series of oxidative stresses or damages in human Healthwise. As shown in table 5, 6,7 plate 11, 12, 13, 14 &15

In view of the limited information about the microbial enzyme (harmless anti-oxidative) of higher altitude, the enzyme played a significant role as an intermediary base between the affected cells of the patients' illnesses in the body and some localized abnormalities for anti-oxidative damages(biosafety). In these interesting results obtained in Table 1. Plate 1, 2, 3 5 &8. However, there were some general which shows microbial population of Six groups i.e. *Bacillus Pasteur, Pseudomonas putida, Enterobacter aerogene, Serratia* sp, *Streptococcus* sp *and Citrobacter freundii*. Thus, were isolated from milk and exposure of home base blood paste. More often a suitable natural enrich medium for both nutrient broth culture and home base broth culture were set up for the production of pharmacological and medicinal therapy using poly herbal plants. Thus, similar events were also conducted from microbial growth in solid agar medium alongside with the home base solid agar medium. These respective bacteria were thereby screen and characterized owing to the nature of their survival at pH 7-8, mode of carbon, nitrogen, assimilation and morphology as seen in table 1 and 2; **Plate 16 &18.** Furthermore, the media were meticulously, assiduously and adequately brought down by acidification to pH 5-6.8 so that the acidophile bacteria might also played a significant role in human good Healthwise once again. Added to these interesting results which were obtained in readiness for protease degrading antioxidant bacterial so as to justified their potential at the initial stage of the research. Further investigation was made through a rigorous buffering of the mild alkaline poly herbal mixture to neutral(pH7) where the acidophile bacteria would also play a significant role to pH 6.78. Thus, this would also convey and identified the performance of various bacteria with the highest capacity to producing substantial drugs discovery like those of HIV/AIDs/ covid-19 and diabetes in a particular fermentation process (either aerobically or facultatively). In quantum mannerism, most of these anti-oxidant's presence in different species of plants extract, the degrading bacteria usually contributed immensely to producing high amount of fermented drugs discovery of medical and pharmacological importance. In view of the perpetual investigation, other interesting features were spotted with respect to the degrading capacity of plant extract by microorganisms to less toxicity for drugs discovery; thus, with regard to antidiabetic properties in human system(biosafety). Added to table 1 once again as shown the microscopic, macroscopic and morphological characteristic were present special features regarding to their shapes gram reaction, spore formation in-situ. Six of the designated or selected bacterial species namely; *Bacillus Pasteur, Pseudomonas putida, Enterobacter aerogene, Serratia* sp, *Streptococcus* sp *and Citrobacter freundii were found to be Rod* (+) and none was spotted with rod (-). Theses respective results were concomitantly agreed or fallen with **[116]** study. However due to their special features spotted in them, they could be more or less stand for the highest of resistant for survival at both mild alkaline and mild acidic under aerobic or facultative condition as seen in **plate** 18. Genetically and metabolically diversity of microorganisms have been used for many years in processes such as antibiotic production, food processing and fermentation etc. Many of such bacteria are set up as application await exploitation for the benefit of human health care and well-being, but depend on the description and study of natural micr0bial wealth. In certain habitats factors conferring selective advantage are obvious. This applied to the mesophile(35^0C-40^OC) tolerance organisms. as seen in table 2; Plate 18. Microorganisms have the ability to adapt to environmental changes. This adaptive capacity may be a reason for the observed physiological flexibility. These kinds of changes may not be genetically conditioned as people always said diabetic have always been in pipe line with heredity when it is not. Probably modification of diabatic could be as well be reversible through the action of these set of bacteria namely; *Bacillus Pasteur, Pseudomonas putida, Enterobacter aerogene etc.,* respectively due to the fact that they possess certain properties by which they differed from the corresponding mesophilic organisms at 35^OC -40^OC as seen in table 2. Similarly, pH is another physical parameter that determine the growth and activities of microorganisms in a given habitat.

Plate 17. posted happy good morning Divine fermented polyherbal medicine (for Diabetic type 1&2) in sterile plastic container in readiness for encapsulation

Herbs are making a comeback and their renaissance is occurring through the world. In today's world, the products derived from herbs signify safety. The synthetic in contrast to herbal products are considered un safe to humans and environment. Herbs extracts have been used as antidiabetic in ameliorating or irradicating antidiabetic, antioxidant, anti-inflammation etc., respectively. In view of the present study as an innovative attempt assessing the micro tolerance level of polyherbal plant extract on some designated or selected organisms like those of *Bacillus Pasteur, Pseudomonas putida, Enterobacter aerogene, Serratia* sp, *Streptococcus* sp *and Citrobacter freundii* as seen in table 3. The poly herbal plant extract mixture was spotted to have some resistivity mutants or properties against some particular organisms with significant

Increasing order of Bacillus sp (+) 0.05<8, Pseudomonas putida (+) 0.05<8 followed by Enterobacter aerogene with decreasing order (-) 0.05> 4 and the lest with the same values were obtained from the following organism in their mode of mutant or properties susceptible in their decreasing orders namely Serratia sp, and Streptococcus sp > Citro *bacter freundii*. The results observed vividly in response to the study of [**117]** once again the interesting results could be possibly and attributed to the synergistic mode of action of phytochemicals present in combination with the mixture of plant extract. Once more could it be that Bacillus ***Pasteur, Pseudomonas putida, Enterobacter aerogene*** exhibited some certain mutational properties that warrant the resistivity of the plant extract to a certain degree of resistance? Certainly, the above highlighted species of organisms would definitely adequately and substantially made hey when the sun shine for reversing the problem encountered with people suffering from perpetual diabetes syndrome without qualm in-situ. In-view of the investigation made by [**118]** that Diabecon Ayush – 82 and Diarun plus65**[119]** that most of polyherbal products produce an excellent natural alternative to artificial or synthetic antidiabetic drugs which have side effect. Plants are the most reliable source and contain a series of potential phytochemical like those of Flavonoid, phenol, alkaloid, saponins, tannins, terpenoid, anthraquinone and plant derived peptides, each of them has shown possible antidiabetic activity in most different experiments **[120].**

Currently, the medicinal plants and herbs are being used in extract forms for their antidiabetic's activity. Various clinical studies confirmed that medicinal plants extracts show antidiabetic activity and restoring the atom of pancreatic beta cell. Results in table 4; as shown polyherbal mixed culture plant extract namely; Beta ***vulgaris*** (beet root), ***Ziniber officinale***(zinger), Moringa leaf/seed (Moringa ***oleifera)***, (***Comellia sinensis***)green tea), Bitter cola(***Garcinia Kola***), (***Mentha***) Mint, Tomato(***Solanum lycopersicum***), Turmeric(***Curcuma longa***), etc., respectively in Table 4, their aqueous seeds (Endocarp), leaves, fruits (Mesocarp) have a lot of antioxidants to the extent that led in reduction of blood glucose level which the artificial drugs like that of allophic drugs do not have. Contrarywise, whole wide large numbers of patients are always treated with natural plant extract and they are always feeling very great and Olympic **[121].** Similar event also agreed more than the expected from Nigerian polyherbal mixed culture capsule drugs produce in the directorate of research and innovation Kaduna polyethnic which has tremendously proven an excellent result as shown in table 4. Thus, the poly herbal drugs made in Nigeria has more or less improved the blood circulation by enlarging the blood vessels and useful in reducing the blood glucose level in the body, it fights against heart disease, pile, it prevents arthritis, it increases antioxidant capacity of the blood (free from Oxidative damage) which is believed to be one of the mechanisms behind aging and many diseases as shown once again in table 4. Furthermore, the Nigeria

poly herbal mixed culture capsule has reported increase in the level of plasma-glucose in diabatic patients are lowered by the capsule supplementation in all group of people within 3 days or so. The antihyperglycemic action resulted from the potentiation of insulin release from the very existing beta cells and regenerating the function of islet cells over time. Nonetheless the poly herbal mixed culture capsule contains so many compounds like those of pimpinellin- dimer, Beta carotenoid, glycosides, tartaric acids, etc., respectively. Thus, they were identified as a potent alpha- glucosidase inhibitor more effective than acarbose as well as significant antioxidants activity. it Is well known that oxidative stress is cause by reactive oxygen species (ROS) and plays an important role in the path genesis of various degenerative diseases such as diabetes. Similarly post prandial oxidative stress is associated with higher risk for diabetes and therefore poly- herbal mixed culture capsule could be tenaciously used as a potent antioxidant and as a strong inhibitor of alpha amylase in the improvement of Type 1 and 2 diabetes patients.

In recent years ethnobotanical and traditional use of natural compound especially those of plant origin has received much attention **as** they are well tested for their efficacies and generally believes to be safe for the treatment of human ailments It's as best classical approach in the search for new molecule for management of the various diseases. Many new bioactive drugs isolated from plants contain mineral, vitamins(supplement) and they showed antibiotic activity that is equally as potent and sometime even more potent than known oral hypoglycemic agents such as metformin and glibendamide.

Plate 18. Posts an expose aerobic fermented polyherbal mixture of plant culture just for a snap shot picture before wrapping for proper fermentation processes

In table 5 have shown encouraging effect polyherbal mixed culture plant extract that have been processed in to capsule for the treatment of diabetic or antidiabetic drugs. Even though the poly herbal mixture culture capsule was harnessed through the isolation of some important constituents of phytochemical analysis like those of Flavonoid, phenol, alkaloid, saponins, tannins, terpenoid, anthraquinone etc., respectively. However, it has proven to be useful in medical and pharmacological drugs recovery for the treatment of diabetes patient in a minimal amount. In view of the current investigations these phytochemical constituents are more or less attributed not only for the treatment of diabetes alone but also act as anti-inflammatory, antioxidant, Anti- preventive properties, Anti- cancer, anti-cold, anti-pneumonia, anti-asthmatic, anti-arthritis, immune booster etc. respectively as shown in table 5. However, it has been reported by **[122]** that the phytochemical constituents gallantly stood as the fighting forces for the suppression of glucose level significantly as well as strong inhibitor of alpha- glucosidase. In a Similar event the Nigeria made polyherbal capsule drugs present an equal or more than some certain properties as inducer of anti-diabetic and antioxidant. Truly speaking the Nigeria made polyherbal capsule drugs has been tested through so many clinical trials to have inhibited alpha glucosidase and decrease glucose transport through the intestinal epithelium. In addition, it contains a lot of secondary metabolites as shown in table 5. This report is in pipeline with the study of **[123].** The Nigeria made polyherbal capsule drugs is attributably and remarkably made to be antidiabetic for the treatment of not only for diabetes patients alone but for so many ailments as reported during the clinical trials. Once again, Nigeria made polyherbal capsule drugs is more of a standard antidiabetic drug than

those of metformin and alloxan-induced diabatic patients. Alloxan monohydrate destroys Beta cells of Langerhans of the pancreas resulting in a decrease in endogenous insulin secretion and pave way for the decreased utilization of glucose by body **[124].** It results in elevation of blood glucose level, decrease protein content increase level of cholesterol and triglyceride(terrible!) **[125]**

Phytochemical screening of aqueous extracts (universal solvent) from polyherbal mixed culture plants from the following Beta ***vulgaris*** (beet root), ***Ziniber officinale***(zinger), Moringa leaf/seed (Moringa ***oleifera)***, (***Comellia sinensis***)green tea), Bitter cola(***Garcinia Kola***), (***Mentha***) Mint, Tomato(***Solanum lycopersicum***), Turmeric(***Curcuma longa***), etc., respectively in Table 6. Thus, taking a quantum survey of both universal solvent (H_2O) and core chemical solvent analysis, the universal solvent appears to have the highest dense (+++) antioxidant properties followed by methanol/ethanol with scanty (++) properties and the least of all was observed on acetone. However, these interesting results must have been in direct pipeline with the study of **[126**]. Although the values obtained by [**127**], were more or less high in comparison to the present investigation or study. Thus, it must likely that the method and values were remarkably, significantly and adequately meant for detection of phytochemical constituents' properties like those of Flavonoid, phenol, alkaloid, saponins, tannins, terpenoid, anthraquinone etc., respectively as shown in table 6 plate…… This promising method employed might vehemently be considered as vehicle for drugs discovery through circumspectly transitioned research, innovation and creativity by human without an oater of bias-ness.

Plants are an important source of phytochemicals which are an important source of drug and medicine. These phytochemicals have extraordinary properties like antibacterial, antifungal, anti-cancerous, antioxidant, anti-inflammatory, anti-diabetic activities etc. The identification of this compound relies on quantitative analysis and hence the knowledge about these techniques is quite important in detecting the designated phytochemical constituent like those of Flavonoid, phenol, alkaloid, saponins, tannins, terpenoid, anthraquinone etc., respectively. Nonetheless further investigation were thereby made through respective combination of plant leaves, seed(mesocarp) and fruit (mesocarp) extract made to obtained a consolidate poly herbal mixed culture capsule for drugs discovery namely; Beta ***vulgaris*** (beet root), ***Ziniber officinale***(zinger), Moringa leaf/seed (Moringa ***oleifera)***, (***Comellia sinensis***)green tea), Bitter cola(***Garcinia Kola***), (***Mentha***) Mint, Tomato(***Solanum lycopersicum***), Turmeric(***Curcuma longa***), etc., respectively in Table 7. These results were taken into recognizance in assessing the potentiality of respective phytochemical screening / antioxidant constituent by their percentages (poly herbal mixed culture medium as shown in table 7. Plate…….. Going by the laid down principle, quantitative estimation of pharmacological important polyherbal/ mixed culture medium, and their constituents were investigated through their properties in percentages they substantially exhibited. Thus, Flavonoid, phenol, alkaloid, saponins, tannins, terpenoid, anthraquinone etc., respectively were duly ascertained for their bioactive properties through which a substantial drug discovery might be achieved. However an interesting results were highly evaluated from the polyherbal mixed culture medium by various, plant, seed(endocarp), fruit(mesocarp) have proven that ***Ziniber officinale***(zinger) and Tomato(***Solanum lycopersicum***) have circumspectly maintained the highest values with almost the same values alkaloid with 16.30 % and 16.2% followed by (Moringa ***oleifera with 15.20%, lime juice (Citrus aurantiifolia), green tea(Comellia sinensis) and Turmaric (curcuma longa***) had averagely with 10.49%, Beet root (Beta Vulgaris) with 13.6% and least were found with almost the same values with 9.8% and 9.7% from Mint (*Metha peperita*)and sugar cane. These varying degrees of differences found in secondary metabolite values from polyherbal mixed culture medium was momentarily in agreement with the findings of [128]. Contrarywise, **[129]** reported less values with 9.2%- 2.4% while **[130]** had previously 12.2- 3.4%. The differences in values obtained might be possibly due to the presence of bioactive compounds accumulated. Consequently, high values content than expected could invariably amount to the lethal nature of plants, microorganisms and humans' health wise care during fermentation as shown in Table 7. What baffled the researcher so much; was could there be any possibility that God has initially and assiduously designed or ear marked the hidden scenarios for existence of plants as well as microorganisms for a synergetic relationship without an oater of qualm? If that evidence is so glaring for humans to have put a second thought, then the existence of plants in their taxa could be a welcome development for humans to tap the efficacies of different phytochemical constituent for medicinal and pharmacological drug discovery. In as much as alkaloid and co played a crucial role as an interferon or antioxidant which is well known in revitalization and resuscitation of the lost glory of insulin production into the body system. Nonetheless, several research works have been geared or done previously and documented by **[131]** for promoting good health to humanity using drug discovery as antidiabetic, anti-inflammatory, antioxidant, anti-allergies etc., respectively. Further investigations were promptly taken into recognizance of the values obtained as shown in table 6 &7. As the case might have been observed by anthraquinone phytochemical constituent properties with regards to following Turmeric ***(curcuma longa***) had meticulously obtained the highest values with 13.5% followed by 12.3% Bitter cola (Garcinia cola), other values were spotted with 10.8% Beet root (Beta Vulgaris), closely related values of 7.8%/9.9% ***Ziniber officinale***(zinger)/Moringa (Moringa oleifera) and the least was 0.3% Tomato (Salmum lycopernum). As a

matter of truth but not the whole truth, the values are in line with the studies of **[132].** However, these research fellows only used Allum gallic as one of the recipes for the treatment of diabatic even though it has some powerful phytochemical constituent for mitigating or ameliorating the problem of diabetes patients. What about the Nigeria made polyherbal mixed culture capsule that contains so many variables like those of the following: Beta ***vulgaris*** (beet root), ***Ziniber officinale***(zinger), Moringa leaf/seed (Moringa ***oleifera)***, (***Comellia sinensis***) green tea), Bitter cola (Garcinia ***Kola***), (***Mentha***) Mint, Tomato (***Solanum lycopersicum***), Turmeric (Curcuma ***longa***), etc respectively. Thus, could the excellent results be compared to the single recipe (Allium garlic)? It is certainly not at all! In view of the current investigation, the poly herbal mixed culture drugs produce in Kaduna polytechnic which have been administered to so many diabetes patient and non-diabetic people in reciprocal, gave a quality reduction of blood sugar level to isotonic point as well as blood pressure too.

Plates 19. posts Divine fermented poly herbal powder in-readiness for encapsule

Before the advent of insulin and oral hypoglycemic/ hyperglycemic drugs, the primary form of treatment involved the use of plants. More than 400 plants have been recommended and recent investigations have been affirmed the potential value of some of these treatments. The hypoglycemic and anti-hypoglycemic effect of several used as anti diabatic remedies has been confirmed. The mode of action or the mechanisms, clinical trials, dosage, manufacture, Diabate level before and after and the effectiveness have been stated in Table 8. According to **[133]** in the recent time, many plants with medical properties were tested for their antidiabetic potentials by various investigators in experiment animals or so. In like those of BGR34, cardifolia, trigonella foerium, Dia-becon, Diasulin etc respectively. On the other hand, Divine fermented polyherbal therapy dosage(capsule) as shown in table 8 was first treated on the experimenter or the researcher as the rat/mice recipe even though he does not have such illness at all (God given therapy). The experimenter (Microbiologist) preferred to die alone so that the medical doctors might not put the blame on him for killing good and abiding citizenry of the country (Nigeria)or internationally. Poly hebal mixed culture capsule therapy produce in Kaduna polytechnic (directorate of research and innovation is an excellent natural alternative to artificial sweeteners, it is a very concentrated microbial medium with only an appreciable amount being ad mistered for 3 consecutive days or so on diabatic patients/ non diabatic people. Poly herbal mixed culture capsule are the most reliable source and contain series of potential phytochemical/ antioxidant like those of alkaloid, glycosides, terpenoid phenolic, flavonoid compound etc., respectively. Poly herbal mixed culture capsule has shown possible antidiabetic, anti-inflammatory, anti-viral, anti-allergies, antimicrobial, antioxidant activities etc., respectively in all ramification of human body. Once again, the poly herbal mixed culture capsule therapy produces in Kaduna polytechnic (Directorate of Research and Innovation have some certain peculiar form of mechanisms in the treatment of diabatic in comparison to others (broad spectrum)

a. Its stimulate cells of the pancreatic islet to release insulin
b. Its resist the hormones which raises the blood glucose
c. its increase the number or raise the potency and sensitivity of insulin receptor site to insulin
d. its decrease the leading out let of glycogen
f. its enhance the use of glucose in the tissue and organ
g. It's clear away free radicals, resist lipid peroxidation and correct the metabolic disorder of lipid and proteins

Table 9.

Clinical survey and share of polyherbal mixed culture capsule at some designated or selected local government areas of Kaduna State like those of Kachia, Kaura, Zangon Kataf, Kajuru, Chikum, Kaduna South, Igabi, Sabon Gari etc., respectively as shown in table 9. The result is in accordance to the study of Silas et al., 2021. Thus, the newly modified therapy is basically not only on diabatic treatment but also on other ailments of interest or importance. Having presented the proposal defense in Kaduna polytechnic at PHC Hall last year before the TEDFUND committees accessing panel; thus, the research fellow was advising to take much of his time for the project; hence, there was an element of truth. The lingering research project regarding to the poly herbal mixed culture capsule therapy was a thing of do or die affairs to the reach of some communities. Lo and behold the most difficult part of the scenarios is when the research fellow mentioned diabetes status. Most men always tried to hide their cockroaches under the carpet when the truth is revealed to them. For the fact that lies travel 1000 time than the truth, and to this effect, the research fellow always received some hint or report on how wonderful the quality of Nigeria polyherbal drug have no side effect irrespective of the differences in their ages. The antidiabetic drug has not given the research fellow a big problem with regard to HIV/AID drugs to the communities. Thereupon the Nigerian polyherbal drugs therapy is not only for diabatic patients but it is also for many illnesses as shown in table9. On the contrary, the share of the Nigerian poly herbal drugs by its clinical trials were distributed to seven local government areas which includes the following; Kachia, Kaura, Zangon kataf, Kajuru, Chikum, kaduna south, Igabi, Sabon Gari and Kubau. Thus, the highest clinical trials were received from 0ver 200persons from Zangon Kataf and Kaduna south, followed by Kaura with over 100 persons and least were spotted from five local government areas with over 50 persons from Kachia, Kajuru, Chikum, Kubau, Sabon gari, and Igabi. Recommendations were highly strong with much gratitude from the villager, and town. Adverse effect received was NIL as well as death toll. On the other hand, both reduction of high blood pressure and glucose level had fallen exactly at isotonic point. By and large could there be causalities what would have happened to the microbiologist by the medical doctors? Certainly, the research fellow would have been crucified or dunghill to nothingness. As a matter of truth, so many people out of hate speech were spotted saying are there evidence of NAFDAC approval? Thank God it was God given medicine. What safe the research fellow from unnecessary hate speech was when they saw the NAFDAC head as an author, on a serious note their heart melted like candle wax before the fire. The approval and recommendation of the Nigerian Polyherbal culture capsule was so glaring by the NAFDAC for a set up standard laboratory for them to come and inspect whether there were quality materials. Thus, this would have provided the avenue for futuristic medium and large-scale drug lap/ factory production, but it appears that everybody wanted the formular. Hope is not lost the research fellow and co are looking forward to the government. ***Breaking New***: For our own information, let us not deceived ourselves regarding to diabatic type I and II, as for the type II diabetes is conditional while the type I diabetes is virus that is responsible (HIV/AIDS).

TABLE 1: IDENTIFICATION AND CHARACTERIZATION OF ISOLATES

acters /	Bacillus Pasteur	Pseudomonas putida	Enterobacter aerogene	Serratia sp	Streptococcus sp	Citrobacter freundii
Umbonate Colony	_	+	_	_	_	_
Trimethylamimine odour	_	+	_	_	_	_
Gram reaction	+	+	+	_	+	+
Rods/cocci	Small Rods chain	Rods	Rods	Rods	Diplococci	Rod
Pigment production	_	+	_	_	+	_
Motility	+	+	_	+	+	_
Swarming	_	_	_	_	_	_
Spore formation	+	_	_	_	_	_
Capsule formation	_	_	_	_	_	_
Aerobic(A)/ Facultatively	A/F.A	A	F.A	F.A	F.A	F.A
Catalase activity	+	+	+	+	_	+
Oxidase activity	+	+	_	_	+	_
Gelatin liquefaction	_	+/-	_	_	_	+
Lipase production	_	_	_	_	_	_
Arginine dihydrolase	ND	+	ND	ND	ND	ND

Indole production	_	_	_	_	+	_
Citrate utilization	_	+	+	+/-	+/-	+
Sodium malonate utilization	+	_	ND	ND	+	_
Urease activity	+	+	+	+	_	_
Denitrification	+	_	+	+	_	_
H_2S-production	-	_	_		_	_
Methyl Red	+	_	+	_	+	+
Voges- Proskauer	+	+	_	+	+	_
Phenylalamine deamination	ND	ND	_	+	_	+
Lysine decarboxylation	ND	_	_	_	+	_
Acid production from glucose	+	+/-	+/-	+	+	+
Acid gas production from	+	+	+	+	_	_
Sucrose	+	+	+	+	+	+
Maltose	+	+				
D-Mannose	+	+	_	_	+	_
Hemolytic Activity	Gamma	Gamma	Gamma	Gamma	Gamma	Alpha

Table 2. PHYSIOLOGICAL GROWTH OR DEGRADERS OF ANTIOXIDANT FOR DRUGS DISCOVERIES

Species names	Tolerant level (pH)	Temperature				
		15	25	35	45	50
Bacillus Pasteur	**7-8**	-	++	+++	+	_
Pseudomonas putida	**6.8 -8**	-	+	+++	+	_
Enterobacter aerogene	**5-7**	-	+	+++	_	_
Serratia sp	**5-7**	-	+	++	_	_
Streptococcus sp	**5-7**	-	++	+++	—	_
Citrobacter freundii	**5-7**	-	++	+++		_

TABLE 3: THE MINIMUM BACTERICIDAL CONCENTRATION OF NIGERIA DEVINE FERMENTED HERBAL MIXTURE (THERAPY) FOR DRUG DISCOVERY AGAINST SELECTED BACTERIAL STRAINS IN-READINESS FOR PHARMACEUTICAL/ INDUSTRIAL/ COMMUNITY USAGE

Strains	Concentration (%V/V)									
	0.05	0.10	0.20	0.30	0.60	1	2	4	6	8
Bacillus Pasteur	+	+	+	+	+	+	+	+	+	+
Pseudomonas putida	+	+	+	+	+	+	+	+	+	+
Enterobacter aerogene	+	+	+	+	+	+	+	_	_	_
Serratia sp	+	+	+	+	+	+	_	_	_	_
Streptococcus sp	+	+	+	+	+	+	+	_	_	_
Citrobacter freundii	+	+	+	+	+	+	+	_	_	_

Key: + Microbial growth: - No growth

Table 4. DIVINE FERMENTED MEDICINAL PLANTS OF NIGERIA ETHNOBOTANY FOR DRUGS DISCOVERY

Scientific names	Common names/ Hausa	Family	Traditional used Phase I	Part of the plants used	Pharmacological used phase II
Garcinia Kola	Bitter kola(Mingora)	Clusiacea	Fight common cold, osteoarthritis, Antibacterial quality, improve immune system, anti-glaucoma, High-blood pressure	Seed	Antidiabetic/ anti-inflammatory
Moringa oleifera	Moringa (Jog ale)	Moringaceae	It's packed with vitamins/ minerals, it helps one's sex life, it helps im-balancing hormone, it protects liver, it helps to fight free radicals,	Leave/seed	Antidiabetic, anti-obesity

			balancing of blood sugar		
Beta vulgaris	Beet-root	_	Improve blood flow, lower blood pressure, it increases exercise performance, prevent cancer, good source of mineral	Root/stem tuber	Antidiabetic
Zingiber officinale	Ginger(chit-ta)	Zingeracaea	Its fight against high blood pressure, heart disease, lung, asthma, pile, it boosts immune system, it helps to prevent blood clots, it reduces the risk of heart and stroke	Rood/stem tuber (Rhizome)	Antidiabetic
Curcuma longa	Turmeric (Gangomo)	Zingeracaea	It prevents arthritis, its increase antioxidant capacity of the Body (oxidative damage) which is believe to be one of the mechanisms behind aging and many diseases, it may low the risk of heart disease	Root/ stem tuber (Rhizome)	Antidiabetic/ anti-inflammatory
Camellia sinensis	Green tea (Kore Shayi)	Theaeae	Its increase fat burning, antioxidant, may lowered the risk of cancer, protect brain aging, may reduce bad breath, it prevents cardiovascular disease and stroke	Leaves	
Mentha	Mint (Mint ganye)	-	It's may fresh one's brain, may improve energy, may fight bacterial infection, it's may improve one's sleep	Leaves	
Tamarindu indica	Tamarind(tsamiya)	Lamiaceae	Good against antiseptic, protect the liver, good for women under pregnancy, weight control, good against constipation and diarrea, its improve one's cardiovascular health	Fruit/seed	
Saccharum officinarum	Sugar Cane (Reke)	Tabaceae	It enhances liver function, it helps fight cancer, it helps to fight hepatitis, it eases the digestive system, it gives a shot of instant energy	Stem	Antidiabetic
Salunum lycoper	Tomoto (Tumatir)	Night -shade	It's good for kidney, good for eyes, it provides essential antioxidant, it lowed blood pressure, it prevents heart attack as well as stroke	Fruit	
Allium sativum .	Gallic (Galiki)	Alliaceae	Combat common cold, may lowered the risk of heart disease, it contains antioxidant that support the body's protective mechanism against oxidative damage, it reduces oxidative stress in those with high blood pressure, at lethetic performance may be improved with supplements	Fruit	
Citrus aurantiifolia	Lime (lemon tsami)	Rutaceae	Vitamin booster, its help to safeguard one's vision, it lowers the risk of Cancer	Fruit	Anti-diabatic

Table 5: DIVINE FERMENTED MEDICINAL PLANTS OF NIGERIA REPORTED CONSTITUENT USE FOR DRUGS DISCOVERY

Scientific/common names	Phytochemical constituents	Pharmacological used	Vitamins	Minerals
Garcinia kola **(Bitter kola)**	Flavonoid, phenol, alkaloid, saponins, tannins, terpenoid, anthraquinone	Antidiabetic, anti-inflammatory, antioxidant, anti-virial properties, immune booster	A, B, D	Mg, Ca, Mn, Iron, P, K
Moringa oleifera **(Moringa)**	Alkaloid, Saponins, Tannins, Steroid, Flavonoid, terpenes Glycoside, phytosterol	Antidiabetic, anti-inflammatory, antioxidant, Anti- preventive properties, Anti-cancer, immune booster	A, B, D, E	Mg, Ca, Mn, Iron, P, K, Cu

Zingiber officinale **(Ginger)**	Saponin, Alkaloid, glycoside, steroid, Flavonoid Terpenoid anthraquinone, tannins	Antidiabetic, anti-inflammatory, antioxidant, Anti- preventive properties, Anti-cancer, anti-cold, anti-pneumonia, anti-asthmatic, anti-arthritis, immune booster	A, B, C, D	Cu, Mg, Mn, Iron, P, K
Curcuma longa **(Turmeric)**	Saponin, Alkaloid, glycoside, steroid, Flavonoid Terpenoid anthraquinone, Tannins	Antidiabetic, anti-inflammatory, antioxidant, Anti- preventive properties, Anti-cancer, anti-cold, anti-pneumonia, anti-asthma, antioxidant, anti-arthritis, immune booster	A, B, D, E	Cu, Mg, Mn, Iron, P, K
Beta vulgaris **(Beet root)**	Phenol Flavonoid, tannins, carotenoid, Alkaloid, Saponins	Antidiabetic, anti-inflammatory, antioxidant, Anti- preventive properties,	A, B,C,D	Mg, Ca, Mn, Iron, P, K, Cu
Comellia sinensis (green Tea)	Saponin, Alkaloid, glycoside, steroid, Flavonoid, Terpenoid, Tannins, terpenes	Antidiabetic, anti-inflammatory, antioxidant, Anti- preventive properties, anti-cold, immune booster	A, B, D	Mg, Ca, Mn, Iron, P, K, Cu
Mentha piperita **(Mint)**	Saponin, Alkaloid, glycoside, steroid, Flavonoid, Terpenoid, Tannins, terpenes	Antidiabetic, anti-inflammatory, antioxidant, Anti- preventive properties, anti-cold, immune booster	A, B, D	Mg, Ca, Mn, Iron, P, K, Cu
Tamarindus indica **(tsamiya)**	Flavonoid, tannins, carotenoid, Alkaloid, Saponins, glycoside, Terpenoid	Antidiabetic, anti-inflammatory, antioxidant, Anti- preventive properties, anti-cold, immune booster	A, B, C, D	Mg, Ca, Mn, Iron, P, K, Cu, Na
Saccharum officinal **(sugar cane)**	Terpenoid, Flavonoid, glycoside, Phenolic acid, steroid, Phytosterols	Antidiabetic, anti-inflammatory, antioxidant, immune booster, diuretic, Anti hyperglycemic	A, B, C, D	Mg, Ca, Mn, Iron, P, K,
Salunum lycoper **(Tomato)**	Carotenoid, Phenolic acid, Flavonoid, glycoalkaloid,	Antidiabetic, anti-inflammatory, antioxidant, Anti proliferative and, antimutagenic Anti- chemo preventive properties, , immune booster	A, B, C	Mg, Ca, Mn, Iron, P, K,
Citrus aurantiifolia(lime)	Flavonoid, Terpenoid, Coumarins, steroid, Terpenoid, Alkaloid	Anti – microbial, anti -cataract, anti-sore throat, anti-chest pain, anti-scurvy, antioxidant,	C, D, E	Mg, Ca, Mn, Iron, P, K,

Table 6: QUALITATIVE ESTIMATION OF PHARMACOLOGICALLY IMPORTANTS SECONDRY METABOLITES CONSTITUENT OF PLANT SPECIES USING AQUEOUS EXTRACTS, ETHANOL METHANOL AND ACETONE FOR DRUGS DISCOVERY

Common/Scientific name of plants	Extraction	Alkaloid	Saponins	Tannins	Steroids	Flavonoid	Glycoside	Terpenoid	Anthraquinone
Bitter-cola (Garcinia cola)	Water	+++	++	++	++	++	+	+++	++
	Methanol	_	+	+	+	+	+		+
	Ethanol	+	_	+	-	-	_		-
	Acetone	-	_	_	+	-	+		_
Moringa leaf/seed (Moringa oleifera)	Water	++	+	++	+	++	++	+++	_
	Methanol	+	+	+	+	_	+	++	_
	Ethanol	+	_	-	+	_	+	+	_
	Acetone	+	_	+	_	+	_	-	_
Ginger (Zingerber officinale)	Water	++	++	++	++	+++	++	++	+++
	Methanol	+	++	+	++	++	++	++	++
	Ethanol	+	+	+	+	+	+	+	+
	Acetone	-	-	+	-	-	-	-	-
Turmeric (Curcuma longa)	Water	+++	++	+++	++	+++	++	++	+++
	Methanol	++	+	++	++	++	+	++	+
	Ethanol	+	+	-	+	++	++	++	+
	Acetone	-	-	-	+	+	-	+	-
Beetroot (Beta Vulgaris)	Water	++	+++	++	+++	++	+++	++	++
	Methanol	+	++	+	-	-	++	+	-
	Ethanol	+	+-	--	+	+	-	+	-
	Acetone	-	-	-	+	-	-	-	+

Green-tea (Comellia sinensis)	**Water**	++	++	+	++	++	++	++	++
	Methanol	+	+	-	+	+	+	+	+
	Ethanol	+	-	+	-	+	-	-	+
	Acetone	-	-	-	+	-	-	+	+
Mint (Mentha Peperita)	**Water**	++	+	++	++	++	++	++	++
	Methanol	+	+	+	+	+	++	+	+
	Ethanol	+	-	-	+	+	+	+	-
	Acetone	-	+	+	-	-	+	-	-
Tamarind (Tamarindus indica)	**Water**	++	++	+++	++	++	++	+++	++
	Methanol	+	+	+	-	+	+	++	+
	Ethanol	+	-	-	+	+	+	+	-
	Acetone	-	-	+	-	-	-	-	+
Sugar cane Saccharum officinal	**Water**	+++	++	+++	++	+++	++	++	++
	Methanol	++	+	+	+	+	+	+	+
	Ethanol	+	_	+	_	+	_	_	+
	Acetone	+	_	_	+	_	+	_	_
Tomato (Salmum Lycoperscum)	**Water**	++	+	+	+	++	+	++	_
	Methanol	+	-	-	_	+	+	+	_
	Ethanol	_	-	-	_	+	-	+	_
	Acetone	_	-	-	_	_	-	_	_
Lime juice (Citrus aurantiifolia)	**Water**	++	++	+++	++	+++	+++	++	++
	Methanol	+	_	++	+	++	+	+	+
	Ethanol	-	+	_	_	+	+	_	+
	Acetone	_	_	_	+	_	_	_	_

Key: +++ abundant; ++ Moderate; + scanty; - no effect

Table 7: QUANTITATIVE ESTIMATION OF PHARMACOLOGICALLY IMPORTANTS SECONDRY METABOLITES IN THE TAXA STUDIED OF PLANT EXTRACTS FOR DRUG DISCOVERY

Taxa	**Alkaloids (%)**	**Saponins (%)**	**Tannins (%)**	**Steroids (%)**	**Flavonoids (%)**	**Glycosides (%)**	**Terpenoids (%)**	**Anthraquinones (%)**
Bitter-cola (Garcinia cola)	**10.20**	**10.3**	**5.6**	**3.9**	**10.3**	**10.2**	**8.9**	**12.3**
Moringa leaf/seed (Moringa oleifera)	**15.2**	**14.5**	**16.5**	**1.2**	**12.4**	**6.3**	**7.5**	7.9
Ginger (Zingerber officinale)	**16.3**	**15.2**	**8.7**	**15.7**	**12.6**	**7.8**	**7.5**	**7.8**
Turmeric (curcuma longa)	**10.3**	**8.9**	**10.5**	**12.6**	**10.2**	**9.2**	**7.8**	**13.5**
Beetroot (Beta Vulgaris)	**13.6**	**9.7**	**7.8**	**9.8**	**9.8**	**7.8**	**7.6**	**10.8**
Green-tea (Comellia sinensis)	**10.6**	**9.8**	**5.6**	**10.0**	**8.9**	**7.8**	**6.9**	**4.7**
Mint (Mentha Peperita)	**9.8**	**7.7**	**6.0**	**9.5**	**7.9**	**7.9**	**7.1**	**4.8**
Tamarind (Tamarindus indica)	**8.5**	**10.7**	**7.8**	**7.9**	**10.3**	**8.3**	**6.8**	**6.2**
Sugar cane Saccharum officinal	**9.7**	**8.9**	**6.5**	**6.2**	**8.9**	**8.4**	**8.5**	**5.3**
Tomato (Salmum lycoperscum	**16.02**	**15.4**	**14.3**	**0.5**	**15.5**	**6.3**	**7.5**	**0.3**
Lime juice (Citrus aurantiifolia	**10.98**	**13.7**	**12.5**	**10.2**	**13.7**	**9.8**	**8.9**	**2.7**

KEY: Maximum Requirements by Bacteria for their survival and antioxidants utilization for the aids of insulin production into various part of the body, microbial proteins, microbial vitamins, microbial mineral played a crucial role in antidiabetic treatment on diabatic patients with less toxic effect to humans

TABLE 8 EFFECTIVE OF POLY HERBAL (MIXTURE), VITAMINS/ MINERALS/ SUPPLEMENT ON DIABATIC PATIENTS: RESULTS FROM A COMMUNITY BASE RESEARCH PROGRAM

Brand name	Dosage	Manufacture	Ingredient	More of action	Clinical trials	Diabatic sugar level		effectiveness	Reference
						before	after		
Divine fermented poly-herbal therapy	Capsule/ sugarcane syrup	Directorate of research and innovation Kaduna poly technic	*Garcinia kola (Bitter kola), Moringa oleifera (Moringa) Zingiber officinale (Ginger), Curcuma longa (Turmeric), Beta vulgaris (Beet root) Comellia sinensis (green Tea), Tamarindus indica (Stamiya), Mentha piperita (Mint), Saccharum officinal (sugar cane), Salunum lycoper (Tomato), Citrus aurantiifolia(lime)*	Stimulate Beta cell of pancreas, reduce insulin, stimulate insulin, insulin resistant, alpha glycoside inhibitor	Over 400 persons	140-185	119-120	Longer than necessary	_
BGR 34 cardifolia , Trigonella foenum graecum, Tinospora cardifolia	Tablet	- Aimil, pharmaceutical	*Berberis aristata, pterocarpus marsupium, Gymnena sylvestre, Rubia cardiofolia*	DPP 4 inhibitor	-	-	-	few weeks	[44]
Dia-becon	Tablet	Himalayan	*Gymnena sylvestre, pterocarpus marsupium, glycyrrhiza glabra, syzygium cumini, boerhavia diffusa, phyllanthus amarus, Tinospora cardifolia, piper nigrum, Ocimum sanctum, triphala, Curcuma longa,*	Insulin, secretogogues , alpha glycosidase inhibitors	-	-	-	Few weeks	[30]
Dia sulin	Tablet	Tobbest busindo	*Cassia auriculata, Coccinia indica, emblica officinalis, Gymnena Promote the sulin sylvestre, Momordica*	Promote the nsulin secrection	---	---	---	Few weeks	[30]

			charantia, syzygium cumini, Tinospora cardifolia, Trigonella foenum graecum						
Bitter gourd	Powder	Garry and Sun Natural remedy	*Momordica charantia*	Reduce insulin resistance	-	-	-	Few weeks	[30]
Dia-care	Powder	Admark herbals limited	*Sanjeevan mool, himej, jambu beej, kadu, namejav, neem chal*	Reduce insulin	-	-	-	Few weeks	
Gurma	Powder	Garry and Sun Natural remedy	*Gymnena sylvestre,*	Maintain blood glucose level	-	-	-	Few weeks	
Diabeta	Tablet	Ayurvedic cure Ayuvedicherbal health productions	*Gymnena sylvestre, vinca rosea, Curcuma longa, Azadirachta indica, beta ayurvedic herbal Tinospora cardifolia, Zingiber officinalis*	Release of insulin	-	-	-	Few weeks	[30]
Pancreas tonic	Liquid	Dr. Morsa cellular botanical	*Tinospora cardifolia, Syzygium cumini, Melia azadirachta, Momordical charantia, Gymnena sylvestre, Aegle marmelos*	Regenerating pancreatic Beta cell	-	-	-	Few weeks	[43]
Sha rangdyab	Powder	Plants medi laboratone pvt. Td	*Green coffee beans, cinnamon, Boerhavia diffuse*	Stimulate insulin production	-	-	-	Few weeks	[23]
Herbal hils jamby	Capsule	Ishaagro developers	*Eugenia Jambolana*	Reduce blood urine sugar level	-	-	-	Few weeks	[23]

TABLE 9: CLINICAL TRIALS AND RECOMMENDATION OFFERED BY DFFRENT COMMUNITIES IN SOME DESIGNATED OR SELECTED LOCAL GOVERNMENT AREAS LIKE THOSE OF KACHIA, KAJURU, ZAGON KATAF, KAURA, CHIKUM, KADUNA SOUTH, IGABI, SABON-GARI ETC RESPECTIVELY FOR THE NEWLY POLYHERBAL DRUGS DISCOVERED

l Government una State)	**cal ls**	**ıgth of nmendation**	**rse effe t**	**r ailments**	**h toll**	**ıction in High l range**	**ıction in iabetic su level e**	**d pulse e**	**gic ion**
ıia	**50 ns**	**ıgly**		**blood pressure brought lown, pile and ulcer arly too**		**120**	**3.1- 3.6**	**l**	
a	**100 ns**	**ıgly**		**blood pressure brought ı, pile and ulcer similarly**		**120**	**3.1- 3.6**	**l**	
on kataf	**200 ns**	**ıgly**		**blood pressure brought ı, pile ad ulcer similarly**		**120**	**3.1- 3.6**	**l**	
ru	**50 ns**	**ıgly**		**blood pressure brought ı, pile and ulcer similarly**		**120**	**3.1- 3.6**	**l**	
un	**50 ns**	**ıgly**		**blood pressure brought ı, pile and ulcer similarly**		**120**	**3.1- 3.6**	**l**	
ına South	**200**	**ıgly**		**blood pressure brought**		**120**	**3.1- 3.6**	**l**	

	ɪns		**ı, pile and ulcer similarly**		
	50	**ıgly**	**blood pressure brought**	**l20**	**3.1- 3.6**
	ɪns		**ı, pile and ulcer similarly**		
Sabon Gari	**50**	**ıgly**	**blood pressure brought**	**l20**	**3.1- 3.6**
	ɪns		**ı, pile and ulcer similarly**		
Kubau	**50**	**ıgly**	**blood pressure brought**	**l20**	**3.1- 3.6**
	ɪn		**ı, pile and ulcer similarly**		

4.2. CONCLUSION

Diabetes mellitus has been considered to be a major cause affecting the economy of patients, their families and society. Furthermore, uncontrolled diabetes leads to serious chronic complications such as blindness, kidney failure, and heart failure, pile, ageing etc., respectively. In order to decrease this problem, researches on new antidiabetic agents are concerned. Because of the adverse effects of modern therapies, many traditional medicines have been noticed. Moreover, herbal extracts nowadays can be converted to a standard poly herbal mixed culture capsule drugs for combinatorial therapies. Each herb has its own active ingredients that can lower blood sugar levels as well as control the complications of diabetes. Future research will focus on isolation, purification, and identification of bioactive substances in plants. This review is looking forward to providing the necessary information in the management of diabetes. In our review, we have introduced a complete list of anti-diabetic plants taken from the Viet herbs database. Isolation and identification of bioactive phytochemicals from these plants play an important role in improving insights into anti-diabetic functional food and drug development

Plate20. posted divine fermented polyherbal drug in capsule (Antidiabetic Medicine)

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