Biochemical Studies of the Ameliorating Effects of Bitter Leaf and Scent Leaf Extracts on Diabetes Mellitus in Humans

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Abstract
The effectiveness of bitter leaf (Vernonia amygdalina) and scent leaf (Ocimum gratissimum) extracts as ameliorating agents of diabetes mellitus disease were studied. A total of 38 diabetic patients were identified within the Rumuepirikom community in Obio Akpor Local Government Area of Rivers state and incorporated into the study. Disease duration in each patient was roughly estimated from the time the disease was clinically diagnosed by medical personnel and all the patients had some basic investigations carried out before the commencement of the study. Each patient was assigned to one of 6 groups based on age of onset of the disease, disease duration in the individual and the severity of the disease in the patient elicited by the presence or signs of complications such as retinopathies, cardiomyopathies, neuropathies, etc.; Their daily fasting blood sugar levels were monitored and extracts of both leaves daily obtained naturally by water extraction were administered to them accordingly. Some groups had the extracts administered alone; some had the extracts given alongside their routine anti-diabetic medication while some served as controls.
Results: *13 patients in the population or 34.21% were females while 25 patients or 65.79% were males. 4 patients or 10.53% had had the disease for a year and less; 7 patients or 18.42% had diabetes for between 1 and 2 years; 11 patients or 28.95% had diabetes for between 3 and 5 years; 13 patients or 34.21% had had the disease for between 5 and 10 years while 3 patients or 7.89% had had the disease for more than 10 years. The disease distribution according to age showed that no patient was aged below 20 years; 1 patient or 2.63% was aged between 21 and 30 years, 2 patients or 5.26% were aged between 31 and 40 years; 7 patients or 18.42% were between 41 and 50 years; 14 patients or 36.84% were between 51 and 60 years; 10 patients or 26.33% are aged between 61 and 70 years; 3 patients or 7.89% were aged between 71 and 80 years and only 1 patient or 2.63% was aged above 80 years. The results obtained showed that patients on both Bitter leaf and scent leaf extracts had good blood sugar control when compared with those that had only one extract administered or those that served as controls and didn't receive the extracts. The administration of the extracts also seemed to mitigate symptoms of neuropathy in afflicted patients over the 1-week period of the study.*

Key Words: *Vernonia amygdalina, Ocimum gratissimum, Diabetes mellitus*

Introduction

Literature Review: Vernonia amygdalina: Bitter Leaf

Vernonia is a genus of about 1000 species of forbs and shrubs in the family Asteraceae. Some of its species are known as ironweed while some are edible and of immense economic value. They are known for having intense purple flowers and the genus was named after the English botanist William Vernon. There are numerous distinct subgenera and subsections in this genus which led some botanists to divide this large genus into several distinct general.

Several species of Vernonia including V. calvoana, V. amygdalina and V. colorata, are eaten as leafy vegetables. Common names for these species include bitter leaf, onugbu in the igbo language, ewuro and ndole. They are common in most West African and Central African countries. They are one of the most widely consumed leaf vegetables of Nigeria, where the onugbu soup is a local delicacy of the igbo people, and of Cameroon, where they are a key ingredient of Ndole. The leaves have a sweet and bitter taste. They are sold fresh or dried, and are a typical ingredient in egusi soup.

Vernonia amygdalina is well known as a medicinal plant with several uses attributed to it, including treatment for diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache and joint pain associated with AIDS (an infusion of the plant is taken as needed). These leaves are exported from several African countries and can be purchased in grocery stores aiming to serve African clients. The roots of V. amygdalina have been used for gingivitis and toothache due to its proven antimicrobial activity.
In Brazil, V. condensata (commonly known as "figatil" or "necroton") is traditionally used as an analgesic, anti-inflammatory, antithermal, antianemic, antibacterial, liver tonic, hepatoprotective, and antiulcerogenic agent.

Vernonia galamensis is used as an oilseed in East Africa. It is grown in many parts of Ethiopia, especially around the city of Harer, with an average seed yield of 2 to 2.5 tonnes per hectare. It is reported that the Ethiopian strains of Vernonia have the highest oil content, up to 41.9% with up to 80% vernolic acid, and is used in paint formulations, coatings plasticizers, and as a reagent for many industrial chemicals.
Vernonia species are used as food plants by the larvae of some Lepidoptera species including Coleophora vernoniaeella (which feeds exclusively on the genus) and Schinia regia (which feeds exclusively on V. texana).

Vernonia altissimum

Vernonia baldwinii

Literature Review: Ocimum gratissimum: Scent Leaf

Ocimum gratissimum, also known as clove basil, African basil and in Hawaii as wild basil is a species of Ocimum. It is native to Africa, Madagascar, southern Asia, and the Bismarck Achilipengo, and naturalized in Polynesia, Hawaii, Mexico, Panama, West Indies, Brazil, and Bolivia. Its other names include Ebe-amwonkho in Edo, Nchuawu in Igbo, Tchayo in Fon [Benin], Daidoya in Hausa, Efinrin in Yoruba, Nunum in Akan, Ntonng in Ibibio and Fobazen in Haiti. Their seeds seem to need strong sunlight to germinate. The essential oil of Ocimum gratissimum contains eugenol and shows some evidence of antibacterial activity. A polyherbal preparation of a water extract obtained from the leaves of Gongronema latifolia, Vernonia amygdalina and Ocimum gratissimum showed analgesic activity. The essential oil has potential for use as a food preservative, and is toxic to Leishmania. Extracts of the leaves are documented to possess anti-diabetic properties, anti-hyperlipidemic effect and recently, it was shown to improve hematological variables in experimental diabetes mellitus via its well reported antioxidant property hence its inclusion in the study.

African Basil: Scent leaf

Scent Leaf

Local Nigerian dish made with scent leaf

A Detoxifying and beautifying Lime and Scent leaf Tea

Literature Review: Diabetic Mellitus

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disease in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination or polyuria, increased thirst or polydipsia, and increased hunger or polyphagia. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, non-ketotic hyperosmolar coma, or death. Serious long-term complications include heart disease, stroke, chronic kidney failure, foot ulcers and retinopathies. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced.

There are three main types of diabetes mellitus:

- Type 1 DM which results from the pancreas's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown.
- Type 2 DM which begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form was previously referred to as "non- insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise.
- Gestational diabetes is the third main form of diabetics and occurs when pregnant women without a previous history of diabetes develop high blood-sugar levels.

Signs and symptoms of Diabetes

Overview of the most significant symptoms of diabetes

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst) and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 DM, while they usually develop much more slowly and may be subtle or absent in type 2 DM. Several other signs and symptoms can mark the onset

of diabetes although they are not specific to the disease and these include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can also cause glucose absorption in the lens of the eyes, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that also occur in diabetes are collectively known as diabetic dermadromes. Diabetic emergencies such as low blood sugar is common in persons with type 1 and type 2 DM. Most cases are mild and are not considered medical emergencies but their effects can range from feelings of unease, sweating, trembling, and increased appetite in mild cases to more serious issues such as confusion, changes in behavior such as aggressiveness, seizures, unconsciousness, and (rarely) permanent brain damage or death in severe cases. Moderate hypoglycemia may easily be mistaken for drunkenness; while rapid breathing and sweating, cold, pale skin are characteristic of hypoglycemia though not definitive. Mild to moderate cases are usually self-treated by eating or drinking something high in sugar while severe cases can lead to unconsciousness and must be treated with intravenous glucose or injections with Glucagon. People (usually with type 1 DM) may also experience episodes of diabetic ketoacidosis, a metabolic disturbance characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness. A rare but equally severe possibility is hyperosmolar non-ketotic state, which is more common in type 2 DM and is mainly the result of dehydration.

Complications: All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other macro vascular diseases are stroke and peripheral vascular diseases. The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. [The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes foot-related problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness. There is a link between cognitive deficits and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function.

Comparison between Type 1 and Type 2 Diabetes

[Feature	Type 1 diabetes	Type 2 diabetes
Onset	Sudden	Gradual
Age at onset	Mostly in children	Mostly in adults
Body size	Thin or normal	Often obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Concordance in identical twins	50%	90%
Prevalence	~10%	~90%

Diagnosis: Glycated hemoglobin and Glucose tolerance test

WHO diabetes diagnostic criteria

Condition	**2 hour glucose**	**Fasting glucose**	**HbA_{1c}**	
Unit	mmol/l(mg/dl)	mmol/l(mg/dl)	mmol/dl	DCCT %
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting Glycaemia	<7.8 (<140)	≥6.1(≥110) & <7.0(<126)	42-46	6.0–6.4
Impaired Glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	42-46	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥48	≥6.5

Diabetes mellitus is characterized by recurrent or persistent high blood sugar, and is diagnosed by demonstrating any one of the following:

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of high blood sugar and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)
- Glycated hemoglobin (HbA_{1C}) ≥ 48 mmol/mol (≥ 6.5 DCCT %).

Prevention: There is no known preventive measure for type 1 diabetes. Type 2 diabetes can often be prevented by maintaining a normal body weight, engaging in physical exercise, and consuming a healthful diet. Dietary changes known to be effective in helping to prevent diabetes include maintaining a diet rich in and fiber, and choosing good fats, such as the polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat and other sources of saturated fats can also help prevent diabetes. Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well.

Management: Diabetes mellitus is a chronic disease for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal, without triggering low blood sugar. This can usually be accomplished with a healthy diet, exercise, weight loss, and use of appropriate medications (insulin in the case of

type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes). Learning about the disease and actively participating in the treatment is important, since complications are far less common and less severe in people who have well-managed blood sugar levels. The goal of treatment is an HbA_{1C} level of 6.5%, but should not be lower than that, and may be set higher. Attention is also paid to other health problems that may accelerate the negative effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet.

Lifestyle: People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and exercise. Also given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure.

Medications: Those used to treat diabetes act by lowering blood sugar levels. There are a number of different classes of anti-diabetic medications. Some are available orally such as metformin, while others are only available by injection such as GLP-1 agonists.
Type 1 diabetes can only be treated with insulin, typically with a combination of regular and NPH insulin, or synthetic insulin analogs. Metformin is generally recommended as a first line treatment for type 2 diabetes, as there is good evidence that it decreases mortality. It works by decreasing the liver's production of glucose. Several other groups of drugs, mostly given orally may also decrease blood sugar in type II DM. These include agents that increase insulin release, agents that decrease absorption of sugar from the intestines, and agents that make the body more sensitive to insulin. When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications. Doses of insulin are then increased to effect. Since cardiovascular disease is a serious complication associated with diabetes, some have recommended that patients maintain blood pressure levels below 130/80 mmHg. The only additional benefit found for blood pressure targets beneath this range was an isolated decrease in stroke risk; however a 2016 review found potential harm to treating lower than 140 mmHg. Among medications that reduce blood pressure, Angiotensin converting enzyme inhibitors (ACEIs) improve outcomes in those with DM while the similar medications, Angiotensin receptor blockers (ARBs) do not. Aspirin is also recommended for people with cardiovascular problems, however routine use of aspirin has not been found to improve outcomes in uncomplicated diabetes.

Materials and Methods

A. Identification of patients

A door-to-door questionnaire was systematically and painstakingly designed and distributed to about 493 households located within the Rumuepirikom area of Port Harcourt, Rivers State.
The questionnaire was designed such that patients with Diabetes mellitus (DM) could give out information about their age, sex, occupation, marital status, age of disease onset which was taken as the age at which the disease was medically diagnosed by a qualified Medical health personnel in a health facility, the number of years the individual had lived with the disease, the current treatment options being used for the patient's management as well as the presence or absence of co-morbidities and complications in the patient.

Compliance to treatment and medication usages as well as lifestyle modifications were painstakingly elicited with the patient's current health status determined before they were in-cooperated into taking part in this four weeks study. The patients were all educated on the

need and purpose of the study and were all given a consent form to sign prior to the onset of the study.
Several visits were done to each identified patient whereby their random and fasting blood sugar tests were often times measured to determine their baseline levels. This was to ensure that the patients were actually diabetic and also to determine the degree of disease present.
The study was done under a four-week study duration in which this period was further sub-divided into two-weekly study periods each. The first 2 weeks were noted as the inactive phase or the preparatory phase whereby the individuals partaking in the study were monitored properly and certain investigations carried out after which they were placed in six groups based on the severity of their diseases, presence of complications or co-morbidities as well as their requirements for daily anti-glycemic agents in order to prevent them for developing serious complications, after which they were all asked to go off their routine medications for one week, being the third week of the study.

Baseline fasting sugar levels were noted for all the patients at the end of 7 days off-medications of the third week.
Other tests that were carried out for them before the commencement of the trial included Liver function tests (ALP, AST), Renal Function tests (Serum Electrolytes, Urea and Creatinine), Eye examinations, Electrocardiography (ECG) and daily fasting and random blood sugars.

B. Collection of Plant Materials:
Fresh leaves of Ocimum gratissimum and Vernonia amygdalina were collected daily for the seven days duration of the study from a home garden located at a private farm situate at Egbelu Community, Rumuolumeni, Port Harcourt, Rivers state.

C. Preparation and Aqueous Extraction of Bitter Leaf Juice:
Freshly plucked bitter leafs (Vernonia amygdalina) were weighed and 300g of the leaves washed in clean water first to remove dirt before they were soaked in fresh water for 2 hours daily. After which they were moderately squeezed to extract the bitter juice from the leaves. The freshly extracted juice was properly stored for use by the patients. The extracts were freshly produced each morning for each patient's use throughout the 7 days period of the active phase of the study.

D. Preparation and Aqueous Extraction of Scent Leaf Juice:
Freshly plucked scent leaves (Ocimium gratissimum) were weighed and 300g of the leaves washed in clean water also to remove dirt before being soaked in fresh water for 2 hours, after which they were mildly squeezed to extract juices from the leaves. The freshly extracted juice were now properly stored for use by the patients. These preparations were also done daily to ensure patients received fresh leaf extracts for the 7 days duration of the study.

E. Phytochemical Analyses of Both Bitter Leaf and Scent Leaf Extracts:
300 g of bitter leaf was soaked in 150 mL of cold water for 24 hours and the resultant juice extracted was air dried and stored. The scent leaf extracts were also prepared using the above procedure.

Qualitative analysis of phytochemical compounds
Qualitative analysis was carried out to ascertain the presence of the different phytochemical compounds contained in the leaves. The extracts were subjected to Thin-layer and paper chromatographic procedures at the laboratory in order to help separate the components into

individual compounds for appropriate identification of all individual components of the extracts.

Quantitative analysis of phytochemical compounds

1. Determination of carotenoids: A measured weight of each sample was homogenized in ethanol using a laboratory blender. A 1:10 (1%) mixture was used. The homogenate was filtered to obtain the initial crude extracts. 20 mL of ether were added to the filtrate to take up the carotenoids mixed well and then treated with 20 mL of distilled water in a separating funnel. The other layer was recovered and evaporated to dryness at low temperature (35-50°C) in a vacuum dessicator. The dry extract was then saponified with 20 mL of ethanolic potassium hydroxide and left over night in a dark cupboard. The next day, the carotenoid were taken up in 20 mL of ether and then washed with two portions of 20 mL - distilled water. The carotenoid extract (ether layer) was dried in a dessicator and then treated with a light petroleum (petroleum spirit) and allowed to stand overnight in a freezer (-10°C). The next day the precipitation steroid was removed by centrifugation and the carotenoid extracts was evaporated to dryness in a weighed evaporation dish, cooled in a dessicator and weighed. The weight of carotenoid was determined was expressed as a percentage of the sample weight.

2. Determination of alkaloids: This was done by the alkaline precipitation gravimetric method described by Harborne. A measured weight of the sample was dispersed in 95% acetic acid solution in ethanol to form a ratio of 1:95(95%). The mixture was allowed to stand for 24 hours. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in weighed filter paper, washed with 1% ammonia solution dried in the oven at 80°c. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

3. Determination of flavonoids: This was determined according to the method of Harborne. 300 gram of the same was boiled in 150 mL of 2 m HCL solution for 30 min under reflux. It was allowed to cool and then filtered through Whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoids precipitated were recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoids in the sample.

E. Grouping of Patients:

After the determination of these diagnostic baselines, the study population was divided into six groups and the study carried out within the remaining seven days of the study.

The six categories were created based on age of onset of disease, disease duration in the individual, presence of complications such as retinopathies, cardiomyopathies, neuropathies, current age of the patient and the baseline fasting blood sugar levels gotten by the 1st week of the active phase of the study.

Group 1

The group was made up of 6 patients whose fasting blood sugar levels prior to onset of the study was between 7.00 to 10.50 mmol/dl or average FBS of 9.00mmol/dl and whose FBs did not vary nor differ much from their total average FBS during the 3 weeks prior to onset of herbal extract usage. They were also noted to be stable and had no signs of diabetic complications, hence were asked not to take any medications for the week of active study in the 2nd fortnight.

Group 2
This group comprised of 6 patients who's FBS were determined to be within the range of 0.51 to 12.50 mmol/dl.
They did not show signs of any complication.

Group 3
This group comprised of 6 patients whose baseline FBS laid between 12.51 to 15.50mmol/dl. They were also stable and had no complications.

Group 4
This group comprised of 6 patients that had baseline FBS of 15.51 to 18.50
Some had signs of diabetic retinopathy with cataract & flame-shaped exudates being predominant.

Group 5
This group included 7 patients whose FBS baseline was between 18.51 to 20.50mmol/dl and average of the elderly patient was incorporated into this group because some of them were noted to have complications such as cardiomyopathies, neuropathies & retinopathy.
I patient had an amputated left leg due to distance diabetic foot ulcer.

Group 6
This group consisted of 7 patients whose baseline FBs was above 20.51mml/dl and who's FBs were triggered up excessively once they went off their prescribed medications.
The 3 Juvenile DM patients were placed in this group with 2 patients noted to have slight nephropathies, Hypertension, with abnormal liver function tests. The 3 juvenile patients were all on insulin injections.

The 1st group (A) was taken as the negative control group whom were not placed on any medication at all for the one week duration of the 2nd phase of the study.

The 2nd group (B) was placed only on bitter leaf extract a glass cup 200mls twice daily for 7 days. No medications.

The 3rd group (C) was placed only on scent leaf extracts a glass cup of 200mls twice daily for 7 days. No medications.

The 4th group (D) was placed on a mixture of both 100mls of bitter leaf and 100mls of scent leaf extracts to make up a glass cup of 200mls twice daily for 7 days. No medications.

The 5th group (E) was asked to take a mixture of both 100mls of bitter leaf and 100mls of scent leaf extracts to make up 200mls daily for 7 days alongside their regular medications.

The 6th group (F) was asked to take only their medically prescribed anti-glycemic agents. Some of these group members were on daily insulin injections.

Results

Table 1: Age Distribution of the Patients (Years)

Age (Years)	Number of patients	Percentage of patients (%)
0 - 10	0	0.00
11 - 20	0	0.00
21 – 30	1	2.63
31 - 40	2	5.26
41 – 50	7	18.42
51 – 60	14	36.84
61 – 70	10	26.33
71 – 80	3	7.89
80 and Above	1	2.63

Table 2: Sex Distribution of the Patients

Sex	Number of patients	Percentage of patients
Male	25	65.79
Female	13	34.21

Table 3: Disease Duration in the Patients (Years)

Duration	Number of patients	Percentage of patients
Less than a year	4	10.53
1 – 2 years	7	18.42
3 – 5 years	11	28.95
>5 – 10 years	13	34.21
>10 years	3	7.89

Phytochemical compounds present in the extracts of washed bitter leaf.

Alkaloids, tannins and steroids were present while flavonoids were absent in the water extract, however when the bitter leaf juice were extracted with alcohol and tested, flavonoids were found to be present, hence flavonoids were assumed not to be present in the bitter leaf extracts used in the study. Tannins were not present in the alcoholic extracts while alkaloids, steroids and flavonoids were present.

Phytochemical compounds present in the extract of washed scent leaf.

Steroids and tannins were absent in the alcoholic extracts and alkaloids and carotenoids present while the water extract was able to liberate the alkaloids, carotenoids and steroids, hence it was assumed that the patients received alkaloids, carotenoids and steroids from the scent leaf extracts during the study.

Table 4: Qualitative screening of washed bitter leaf extracts

Mode of Extraction	Alkaloids	Flavonoids	Steroids	Tannins
Water Extraction	+	-	+	+
Alcohol Extraction	+	+	+	-

Table 5: Qualitative screening of washed scent leaf extracts

Mode of Extraction	Alkaloids	Carotenoids	Steroids	Tannins
Water Extraction	+	+	+	-
Alcohol Extraction	+	+	-	-

Table 6: Quantitative screening of washed bitter leaf extracts in %(g/100g).

	Alkaloids	Flavonoids	Tannins
Alcoholic Extraction	2.91	2.30	-
Water Extraction	6.73	--	0.18

Table 7: Quantitative screening of washed Scent leaf extract in %(g/100g)

	Alkaloids	Carotenoids
Alcoholic Extraction	2.33	0.17
Water Extraction	1.98	0.06

Discussion

The effectiveness of bitter leaf (Vernonia amygdalina) and scent leaf (Ocimum gratissimum) extracts as curative agents of diabetes mellitus disease were studied. Of the 38 patients that were involved in the study, 13 patients or 34.21% were females while 25 patients or 65.79% were males (Table 2). 4 patients or 10.53% had had the disease for a year and less; 7 patients or 18.42% had diabetes for between 1 and 2 years; 11 patients or 28.95% had diabetes for between 3 and 5 years; 13 patients or 34.21% had had the disease for between 5 and 10 years while 3 patients or 7.89% had had the disease for more than 10 years (Table 3). The disease distribution according to age showed that no patient was aged below 20 years; 1 patient or 2.63% was aged between 21 and 30 years, 2 patients or 5.26% were aged between 31 and 40 years; 7 patients or 18.42% were between 41 and 50 years; 14 patients or 36.84% were between 51 and 60 years; 10 patients or 26.33% are aged between 61 and 70 years; 3 patients or 7.89% were aged between 71 and 80 years and only 1 patient or 2.63% was aged above 80 years (Table 1). At the end of the study, results obtained showed that patients on both Bitter leaf and scent leaf extracts combined (Group D) had good blood sugar control when compared with those that had only one extract administered solely (Groups B and C) or those that served as controls and didn't receive the extracts at all (Group A). Patients that received doses of single extracts alone (Groups B and C) also had better controlled sugar level results than those who did not receive extracts at all (Groups A and F). Also, patients who took the extracts alongside their routine medication (Group E), had better sugar level control than those who took their medications alone without the extracts (Group F). Comparison between Group B and Group C patients revealed that those who received bitter leaf extracts alone (Group B) had better sugar level stability than those that received scent leaf extracts alone (Group C).The administration of the extracts also seemed to mitigate symptoms of neuropathy in afflicted patients over the 1-week period of the study.

Conclusion

The above results revealed that both bitter leaf and scent leaf extracts had moderately significant ameliorative effects on diabetes mellitus disease and worked more effectively when combined together. Single administrations also proved to be better than non-administration of either. Also, the combination of these extracts with normal routine medications drastically reduced sugar levels in patients when compared with only routine

medication usage. Bitter leaf extracts probably had higher ameliorative effects than scent leaf extracts probably because of it alkaloid and tannin components which were extracted by aqueous extraction as patients took only aqueous extracted juice to avoid consuming toxic compounds associated with alcoholic extractions.

Ethics

Patients that partook in this study were all duly informed and educated on the reasons for the study as well as informed consent forms administered to them for their signing. Care was taken during preparation and storage of the extracts to ensure that the extracts were prepared under good hygienic standards. They were freshly prepared daily in order to prevent contamination, hence preventing diseases and unwanted side effects from their consumption.

Recommendations

We wish to recommend that further work be done to determine precisely how effective bitter leaf and scent leaf extracts could be in the management of diabetes mellitus as well as other ailments.

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