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Phytochemical Analyses and *in vitro* Anti-Diabetic Activity of Ten Indigenous Plants

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ABSTRACT

Background and Objective: Diabetes is a major global health concern affecting humans worldwide. The people prefer natural remedies to conventional medicines in Nigeria. In our ongoing exploration of indigenous plants to treat endemic diseases, the study investigated ten locally grown vegetables commonly used in Akwa Ibom State, Nigeria for the treatment of diabetes mellitus to authenticate their use for the treatment. **Materials and Methods:** The phytochemicals were analyzed qualitatively and quantitatively using standard methods; while the anti-diabetic activity was assayed using glucose uptake by yeast method with percentage calculation per extract. Results were statistically analyzed using One-way Analysis of Variance (ANOVA) at $p>0.05$ level. **Results:** The study indicated the presence of flavonoids, tannins, phenols, alkaloids and saponins in good concentrations in all the plants examined. The results also revealed that there was an increase in glucose uptake by yeast when the ethanol extracts of these plants were introduced. **Conclusion:** The results indicated the ability of these extracts to enhance glucose utilization showing that they can lower blood glucose levels. This also highlighted their potential use in treating and managing diabetes.

KEYWORDS

Phytochemical analysis, anti-diabetic activity, indigenous plants, ethanol extract, blood glucose level management

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by elevated levels of glucose in the blood (hyperglycemia) and insufficiency in the production or action of insulin produced by the pancreas in the body[1]. Insulin is a hormone synthesized by beta cells in the pancreas in response to various stimuli such as glucose, sulfonyl urease and argine; however, glucose is the main determinant[2]. According to the American Diabetes Association (ADA)[3] and Diabetes Care[4,5] diabetes can be classified into four general groups, namely-Type 1 diabetes; which occurs due to beta cell destruction, usually leading to absolute insulin deficiency. It could also be called insulin dependent diabetes mellitus; Type 2 diabetes; which occurs due to progressive insulin secretory effect on the background of insulin resistance. The body can produce insulin but cannot utilize it. It could also be called non-insulin dependent diabetes mellitus or gestational diabetes mellitus (GDM)-this is experienced in the second or third trimester of pregnancy but is not open and observable diabetes; the other type of diabetes is due to other causes; for example,

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monogenic diabetes syndromes like neonatal diabetes and maturity-onset diabetes of the young, diseases of the exocrine pancreas (such as cystic fibrosis), Drug- or chemical-induced diabetes; such as in the treatment of AIDS or after organ transplantation.

Oxidative stress and hyperlipidemia have been discovered to also play important roles in the development of diabetes mellitus. It is believed that oxidative stress is involved in the development of vascular complications in diabetes mellitus particularly type 2[6]. Oxidative stress is usually caused by free radicals in the body. Free radicals are short-lived chemical entities containing one or more unpaired electrons. They exert damage by passing the unpaired electrons to the cell resulting in oxidation of the cells components and molecules[7].

Other factors that play important roles in the development of diabetes mellitus include-weight (the fattier tissues you have, the more resistant your body is to insulin), inactivity (the less active you are, the greater your risk of developing diabetes mellitus), family history, race or ethnicity, age and polycystic ovary syndrome, etc.

Locally derived plants have been an important source of medicinal agents for the management of diabetes. The use of herbal medicine in the management of diabetes mellitus is based on the premise that plants contain active ingredients that possess pharmacological activities responsible for the treatment of illnesses, promotion of good health and boosting of the immune system. Some of these active ingredients include flavonoids, tannins, terpenoids, triterpenoids, sapogenins, alkaloids, vitamins and saponins, etc. Each plant could contain one or a group of these active ingredients making it suitable for the treatment of a wide variety of illnesses.

In most African countries, especially Nigeria, people prefer to use natural products to treat and manage this disease instead of the conventional anti-diabetic drugs available in pharmacies. This is because of the cost, side effects and adulteration associated with the use of conventional anti-diabetic drugs. This study investigated the phytochemical compositions and *in vitro* anti-diabetic properties of ethanol extracts of the ten different locally derived plants namely: *Abelmoschus esculentus*, *Allium sativum*, *Aloe vera*, *Jatropha curcas*, *Mangifera indica*, *Ocimum gratissimum*, *Psidium guajava*, *Telfairia occidentalis*, *Vernonia amygdalina* and *Zingiber officinale*.

MATERIALS AND METHODS

Study duration and location: The study was conducted in the Research Laboratory of Pharmaceutical and Medicinal Chemistry, Department of Faculty of Pharmacy, University of Uyo between June, 2022 and June, 2023. Uyo, the capital of Akwa-Ibom State of Nigeria with coordinates 5°2'N and 7°55'E is located within the equatorial region; about 60 km from the coast of the Atlantic Ocean.

Materials

Equipment: Water bath (selecta, Spain), weighing balance (S. Mettler), UV-Visible spectrophotometer (Labon Med Inc.), extraction tanks, centrifuge.

Solvents, reagents and chemicals: Distilled water, ethanol, methanol (James Burrough Limited), Mayer's reagent, Dragendroff's reagent, glucose, acetic acid, magnesium metal, concentrated sulphuric acid, ferric chloride, aluminum chloride sodium hydroxide, sodium nitrite and acetone (BDH Chemicals Limited).

Methods

Collection and identification of plant materials: Ten plant materials were obtained from Uyo main market, botanical gardens and farmlands in Uyo Akwa Ibom State. All the plants were identified by

Dr. Imoh I. Johnny of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo and assigned voucher numbers in the Herbarium as follows: *Abelmoschus esculentus* (UUPH A9a), *Allium sativum* (UUPH 44b), *Aloe vera* (UUPH A9a), *Jatropha curcas* (UUPH 44b), *Mangifera indica* (UUPH A9a), *Ocimum gratissimum* (UUPH 38a), *Psidium guajava* (UUPH A9a), *Telfairia occidentalis* (UUPH 28d), *Vernonia amygdalina* (UUPH 10j) and *Zingiber officinale* (UUPH 80l).

Extraction procedures: This was done by maceration using 70% ethanol. The ground leaves, fruits and roots of the different plants were weighed and put into an extraction tank. Then 1 L of 70% ethanol was added to each of the tanks containing the plant part, shaken, covered and allowed to stand for 3 days with intermittent shakings. After 3 days, the mixture was filtered and the filtrate was evaporated under reduced pressure at room temperature to obtain dry extracts of each of the plants' parts.

Qualitative phytochemical screening: Chemical tests were carried out on the aqueous extracts of each of the plant materials to identify the phytochemical constituents using the standard procedures as described by Evans and Trease[8] and Sofowora[9].

Quantitative determination of phytochemicals

Determination of total alkaloids: The supernatant of the aqueous extract (5 mL) was dissolved in 5 mL 0f 0.1NHCl in a flask, shaken thoroughly for 2-3 min and allowed to stand to separate. The lower layer of 5 mL was titrated with 0.1 NNaOH till the colour changed from red to yellow. The total alkaloid was calculated using; 1 mL 0.1 NHCl = 0.0612 g of alkaloid[10].

Determination of total flavonoids: The aqueous extract (0.3 mL) was added to methanol (30%, 3.4 mL) with $NaNO_2$ (0.15%, 0.5 mL) and $AlCl_3.6H_2O$ (0.3M, 0.5 mL), mixed and allowed to stand for 5 min; after which 1 mL 1 M NaOH was added and kept at room temperature for 30 min. The absorbance was taken at 506 nm using UV/Visible spectrum in triplicate. The total flavonoid content was calculated using the standard graph of quercetin and the result was expressed as quercetin equivalent (mg/g)[10].

Determination of total phenolics: The extract (1 mL) was added to Folin-Ciocalteus's reageant (1 mL), Na_2CO_3 (2 mL) and distilled water (1 mL) and mixed thoroughly for 5 min. The mixture was incubated for 90 min at 25°C and the absorbance was taken at 750 nm. The total phenolic content was determined from Beer-Lambert's law and using a calibration curve of gallic acid solution expressed as milligram of gallic acid equivalent of a dried sample[10].

Determination of total saponins: Extract (0.5 g) was dissolved in distilled water shaken vigorously and allowed to stand for 1 hr. A formation of a stable foaming froth was observed. As 1 mL of the mixture and 1 mL of olive oil were mixed and shaken to obtain a cloudy appearance. The absorbance was measured at 620 nm using a spectrophotometer in triplicate. The total saponin content was calculated using the standard graph of saponin[10].

Determination of total tannins: Extract (0.5 g) was dissolved in distilled water and 2.5 mL of filtrate and 1 mL of 0.1MHCl in drops were added with 0.3 mL $K_4Fe(CN)_6.3H_2O$. The absorbance was measured at 395 nm. The result was expressed in terms of tannic acid (mg/g) using the standard tannic acid graph[11].

Extraction of the drug (glibenclamide): The drug, glibenclamide (Brand name: Clamide by Hovid) was crushed in a mortar (20 tablets). The milled drug was then extracted with 250 mL acetone, filtered and the filtrate allowed to dry at room temperature. The exact weight of the drug extract was recorded.

Preparation of the drug

Extraction of the standard drug: The extracted drug (glibenclamide, 0.60 g) was dissolved in 60 mL of distilled water to give a 10 mg/mL concentration.

Preparation of glucose: A stock solution of 1% glucose solution was prepared by dissolving 0.1 g of glucose in 100 mL of the aqueous solution.

Preparation of extracts: Each of the extracts (0.2 g) was dissolved in the solvent used for extraction to give a 10 mg/mL concentration.

Estimation of yeast uptake: A stock solution of 1% glucose was prepared and different dilutions of 10, 20, 30, 40 and 50 µg/mL were prepared in different test tubes. As 1 mL of yeast solution was transferred into each of the test tubes and vortexed. To another set of 5 test tubes containing different dilutions of glucose concentration (10, 20, 30, 40 and 50 µg/mL), 1 mL of yeast solution was added and vortexed and then 1 mL of 70% ethanol extract was then added to each test tube and incubated in a dark cupboard for 60 min at room temperature. The absorbance was taken at 540 nm using a UV-visible spectrophotometer. The procedure was repeated for each of the extracts and the drug.

The percentage increase in glucose uptake by yeast was calculated using the formula below[12]:

$$\text{Increase in glucose uptake}\,(\%) = \frac{ABS_{control} - ABS_{sample}}{ABS_{control}} \times 100$$

Where:

$ABS_{control}$ = Absorbance of the control reaction that does not contain the extract or drug

ABS_{sample} = Absorbance of the test samples

All experiments were carried out in triplicates.

Statistical analysis: All results were expressed as Mean±SEM and were analyzed by One-way Analysis of Variance (ANOVA) using MS Excel 2013. p 0.05 was taken as significant.

RESULTS AND DISCUSSION

Phytochemical analysis: The qualitative phytochemical screening showed that all the plants contained alkaloids, saponins, tannins, flavonoids and phenolic compounds as shown in Table 1. The percentage compositions of all the phytochemical compounds present in each of the plant extracts are shown in Table 2.

Antidiabetic activity evaluation-glucose uptake by yeast assay: The percentage of glucose uptake by yeast which represents the antidiabetic activity of each extract of the plants is shown in Table 3.

The phytochemical screening showed that the ethanol extracts of all ten plants contained alkaloids, flavonoids, phenols, saponins and tannins (Table 1 and 2). This confirmed the findings previously made[13-17].

The ethanol extracts of all the ten vegetables examined showed various degrees of percentage increases in the glucose uptake by yeast with all the concentrations used. This showed that they possessed some level of antidiabetic activity (Table 3). This aligned with the information provided by previous researchers on these plants[17-19].

Table 1: Qualitative phytochemical screening of the plant extract

Plant	Common name	Alkaloid	Saponin	Tannin	Flavonoid	Phenol
Abelmoschus esculentus	okra	+	+	+	+	+
Allium sativum	Garlic	+	+	+	+	+
Aloe vera	Aleo vera	+	+	+	+	+
Jatropha curcas	Hospital too far	+	+	+	+	+
Mangifera indica	Mango	+	+	+	+	+
Ocimum gratissimum	Scent leaf	+	+	+	+	+
Psidium guajava	Guava	+	+	+	+	+
Telfairia occidentalis	Fluted pumpkin	+	+	+	+	+
Vernonia amygdalina	Bitter leaf	+	+	+	+	+
Zingiber officinale	Ginger	+	+	+	+	+

+: Present and -: Absent

Table 2: Quantitative evaluation of the phytochemical constituents of the ten plants

	Composition of the phytochemicals in the extracts (%)				
Plant/vegetable	Flavonoids (%)	Phenolics (%)	Saponins (%)	Alkaloids (%)	Tanins (%)
Abelmoschus esculentus	0.02	0.28	0.27	0.02	0.11
Allium sativum	0.11	0.30	0.30	0.02	0.11
Aloe vera	0.01	0.24	0.23	0.02	0.10
Jatropha curcas	0.01	0.29	0.28	0.01	0.11
Mangifera indica	0.02	0.83	0.81	0.02	0.10
Ocimum gratissimum	0.01	0.83	0.82	0.02	0.11
Psidium guajava	0.25	0.85	0.47	0.02	0.13
Telfairia occidentalis	0.07	0.77	0.75	0.01	0.13
Vernonia amygdalina	0.02	0.30	1.03	0.03	0.15
Zingiber officinale	0.03	0.52	0.50	0.02	0.11

Table 3: Percentage increase in glucose uptake by yeast for the extracts and glibenclamide at different glucose concentrations

Extracts	10 µg/mL uptake (%)	20 µg/mL uptake (%)	30 µg/mL uptake (%)	40 µg/mL uptake (%)	50 µg/mL uptake (%)
Abelmoschus esculentus	43.8	54.2	59.6	65.1	69.0
Allium sativum	46.4	47.8	51.5	58.8	63.2
Aloe vera	10.7	39.0	51.0	67.0	69.0
Jatropha curcas	88.8	90.2	89.1	89.3	89.2
Mangifera indica	71.9	79.6	79.5	81.1	81.6
Ocimum gratissimum	63.7	66.1	66.5	73.2	77.4
Psidium guajava	92.5	93.8	93.7	94.0	94.0
Telfairia occidentalis	66	67.3	75.8	78.7	82.3
Vernonia amygdalina	79.2	81.9	84.3	93.4	94.2
Zingiber officinale	25.0	48.8	55.6	63.9	75.3
Glibenclamide	55.0	65.2	76.7	81.4	82.5

Phytochemicals are responsible for the pharmacological and biological activities of plants. Glucose uptake by yeast is usually by facilitated diffusion. Recent information on the transport of sugars across the yeast membrane shows that transport across the membrane is mediated by stereospecific membrane carriers. These carriers transport solutes down the concentration gradient indicating that effective transport is achieved if there is removal of intracellular glucose. So, glucose transport occurs only if the intracellular concentration of glucose is reduced (utilized)[20].

The results from the study of these extracts showed that the plants were able to enhance glucose uptake, hence enhancing glucose utilization, thereby controlling blood glucose levels. The plant with the highest activity from this study was *Vernonia amygdalina*. This research has shown that these locally derived plants have phytochemicals that possess anti-diabetic properties. This is useful in traditional and herbal medicine practice and also useful for patients who prefer treatment with herbs and should be used as part of their diet. Future efforts should be directed at isolating these active compounds and optimizing their activity as drug products for treatment and management of diabetic cases.

CONCLUSION

The research sheds light on the anti-diabetic properties of various edible leaves. Among the tested plant extracts, *Vernonia amygdalina* stands out as the most potent, followed by *Psidium guajava* and *Jatropha curcas*. These findings underscore the potential of these natural remedies in diabetes treatment and management. Furthermore, it is crucial to explore these plant extracts to investigate their active compounds and consider integrating them into complementary therapies for diabetes. Additionally, promoting awareness about their benefits among healthcare practitioners and patients can enhance their utilization. In summary, these humble leaves, often enjoyed as delicacies, hold promise as valuable allies in the fight against diabetes.

SIGNIFICANCE STATEMENT

This research work aimed to find out the quality and quantity of phytochemicals and anti-diabetic potentials of ten locally obtained and consumed vegetables in Uyo, Akwa Ibom State, Nigeria. The study discovered all the samples analyzed possessed significant levels of dose-dependent anti-diabetic activity as the extent of glucose uptake increased with an increase in the concentration of the extracts. Thus the research has highlighted the anti-diabetic potentials of these locally obtained plants which were not established by previous researchers. This is a useful source of information for future researchers and encouragement to the local consumers of the vegetables especially those with diabetic cases as constant and continuous consumption may enable good management of the cases.

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