Original Article

Phytochemical Analysis, *in vitro* Antioxidant Activity and Inhibition of Key Diabetic Enzymes by Selected Nigerian Medicinal Plants with Antidiabetic Potential

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ABSTRACT

Background: Diabetes is one of the most common metabolic disorders worldwide and the disease is generally believed to be incurable. However, the historical success of natural products as therapeutic agents has led to the search for more herbs with antidiabetic potential. **Aim:** To investigate the antioxidative and antidiabetic activities of some common medicinal plants traditionally used to treat diabetes in Nigeria. **Materials and Methods:** Twenty one plant samples comprising leaves, seeds and stem bark were separately extracted using three solvents of increasing polarity namely ethanol, methanol and distilled water. The extracts were subsequently screened for their phenolic profile, ferric reducing power as well as inhibition of α-amylase and haemoglobin glycosylation activities as markers of *in vitro* antidiabetic activity. **Results:** Solvents extracts of 7 out of the 21 plants examined namely *Anacardium occidentale* (leaf), *Carica papaya* (leaf), *Ficus asperifolia* (leaf), *Hibiscus sabdariffa* (stem bark), *Khaya senegalensis* (stem bark), *Ocimum gratissimum* (leaf) and *Parkia biglobosa* (stem bark) had significant concentrations of total polyphenol and flavonoid compared to the other plants. Similarly, for all the *in vitro* models used in this study, the same set of plants demonstrated significantly ($p < 0.05$) higher effects which were comparable to ascorbic acid, acarbose and gallic acid. **Conclusion:** Findings from this study indicate strong correlation between polyphenolic concentration and antidiabetic activity. The study also confirmed the potential of some Nigerian herbs in antidiabetic drug discovery; and identified a number of promising plants for further *in vivo* investigation as plant-based antidiabetic agents.

Key words: Diabetes, Antioxidant, α-amylase, Haemoglobin glycosylation, Natural products.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both.[1] It is a common and prevalent disease affecting people in both developed and developing countries. Worldwide, the prevalence of diabetes mellitus is on the rise with a projected 5.4% increase by 2025.[2] According to International Diabetes Federation, there are about 387 million people afflicted with diabetes worldwide, with 22 million residing in sub-Saharan Africa. Nigeria is the most populated country in Africa and is home to 4 million people with diabetes, representing a fifth of all diabetes cases in sub-Saharan Africa. More worrisome is the fact that about 70%-80% of the diabetes cases in Nigeria remain undiagnosed or untreated.[3] According to the most recent information on the prevalence of diabetes in South Africa,[4] about 6% of the population have confirmed and latent cases of diabetes with a possible sharp rise in this number in the nearest future. Despite these alarming statistics, diabetes still remain one of the age long chronic diseases of human race and its frontiers are expanding by the day.

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On the basis of aetiology and clinical presentation, diabetes mellitus is classified into two namely type 1 and type 2 diabetes. Type 1, known as insulin–dependent diabetes mellitus (IDDM) is caused by immunological destruction of pancreatic β cells resulting in insulin deficiency.[5] It is mainly triggered by environmental factors that may activate autoimmune mechanisms in genetically susceptible individuals, leading to progressive loss of pancreatic islet β cells.[6] Type 2 diabetes, also known as non-insulin–dependent diabetes mellitus (NIDDM), is characterized by both impaired insulin secretion and insulin resistance; and it is often associated with sedentary lifestyle, obesity and hereditary disposition.[7]

Recently, free radical generation has been suggested to play an important role in the cause and complications of diabetes.[8] These radicals are continually produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. In healthy individuals, generation of free radical appears to be approximately in balance with the antioxidant defense system comprising both enzymatic and non-enzymatic antioxidants. In diabetic state however, there are alterations in the endogenous free radical scavenging mechanisms which may lead to the production of reactive oxygen species, resulting in oxidative damage and tissue injury.[9] The implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen-free radicals but also due to non-enzymatic protein glycosylation and auto-oxidation of glucose,[10] alterations in antioxidant enzymes[11] as well as formation of lipid peroxides.[12] Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes, are thought to be the etiology of diabetic complications.[12]

The management of diabetes is considered a global problem and the search for a definite therapy is still ongoing. A few chemotherapeutic drugs have been used to manage the disease; however the application of these drugs is limited due to their high cost and associated side effects. In the last few years, attention is being focused on the identification of natural products from plants to replace the synthetic drugs. Consequently, there has been an exponential growth in the use of herbal medicine because of its natural origin and less side effects.[13] This approach continues to play an important role in diabetic therapy, particularly in developing countries where many people have limited resources and do not have access to modern treatment.[14]

In Nigeria, the list of plant species identified for the treatment of diabetes based on traditional folklore medicine is endless; particularly in the Central, Eastern and Western regions which are recognized as the richest flora communities in Nigeria. Unfortunately, there is no scientific basis for the use of many of such plants as antidiabetic agents. Therefore, the present study was designed to investigate the *in vitro* antidiabetic activity of different parts of 21 commonly used medicinal plants in Nigeria extracted using three solvents namely ethanol, methanol and distilled water. This is of great importance in order to lend scientific credence to the use of the herbs for the treatment of diabetes by traditional healers.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteau reagent, hexane, methanol and ethanol were products of Merck Chemical Company (Darmstadt, Germany). Ascorbic acid, tannic acid, quercetin, hemoglobin, gallic acid, aluminum chloride, α-amylase and ferric chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gentamycin was purchased from EMD-Chemicals, San Diego, CA, USA. All other chemicals and reagents were of analytical grade.

Plant materials

Twenty one medicinal plants comprising leaves, seeds and stem bark were freshly collected in November, 2016 from local villages across Kwara, Kogi and Ekiti States in Nigeria, West Africa. The plants and parts are *Alchornea cordifolea* (leaf), *Anacardium occidentale* (leaf), *Anthocleista djalonensis* (leaf), *Bridelia ferruginea* (leaf, seed and stem bark), *Carica papaya* (leaf), *Ceiba pentandra* (stem bark), Citrus *paradisi* (leaf), *Cnestis ferruginea* (leaf), *Ficus asperifolia* (leaf), *Ficus exasperata* (leaf), *Gongronema latifolium* (leaf), *Hibiscus sabdariffa* (stem bark), *Hunteria umbellata* (seed), *Indigofera pulchra* (leaf), *Khaya senegalensis* (leaf and stem bark), *Mangifera indica* (leaf and stem bark), *Morinda lucida* (leaf), *Nauclea latifolia* (leaf), *Ocimum gratissimum* (leaf), *Parkia biglobosa* (stem bark) and *Viscum album* (leaf and seed). The plants were identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria by Mr. Bolu Ajayi and tagged with appropriate voucher numbers (Table 1). Specimen samples were thereafter prepared and deposited accordingly at the Herbarium of the University.

Preparation of plant samples

The plant samples were treated as previously described.[15] Briefly, the samples were thoroughly rinsed under running tap water followed by distilled water to cleanse them of dirt, dust and debris. They were thereafter shade-dried for two weeks to constant weights. The

Table 1: Plants Studied with Voucher Number.

Plants	Voucher Number
Alchornea cordifolea	UILH/002/666
Anacardium occidentale	UILH/003/612
Anthocleista djalonensis	UILH/001/1248
Bridelia ferruginea	UILH/002/988
Carica papaya	UILH/003/945
Ceiba pentandra	UILH/001/957
Citrus paradisi	UILH/001/995
Cnestis ferruginea	UILH/001/1034
Ficus asperifolia	UILH/001/1210
Ficus exasperata	U1LH/001/883
Gongronema latifolium	UILH/002/1196
Hibiscus sabdariffa	UILH/003/646
Hunteria umbellata	UILH/001/1151
Indigofera pulchra	ULLH/002/421
Khaya senegalensis	UILH/001/852
Mangifera indica	UILH/003/490
Morinda lucida	UILH/002/1103
Nauclea latifolia	UILH/002/806
Ocimum gratissimum	UILH/003/984
Parkia biglobosa	UILH/003/313
Viscum album	UILH/001/1210

dried samples were milled to a fine powder and then stored separately in airtight containers for transport to Cape Peninsula University of Technology, Wellington Campus, South Africa for further analyses.

Preparation of plant extracts

Using 200 mL of hexane, 30 g each of the powdered samples were defatted before they were separately extracted with 200 mL of ethanol, methanol and distilled water by soaking for 48 h. This was followed by 2 h orbital shaking at 200 rpm to allow for maximum extraction before filtration using Whatman No. 1 filter paper. The ethanol and methanol extracts were evaporated at 40°C under reduced pressure using a rotary evaporator while the distilled water extract was dried on a water bath at 45°C. The resulting extract in each case was then weighed and transferred into sample bottles for storage in a refrigerator at 4°C until required for further analyses.

Estimation of total polyphenol content

The total polyphenol content of each extract was determined according to the method described by Wolfe *et al.*[16] with slight modifications. Briefly, 200 µL of each solvent extract (240 µg/mL) was incubated with 1 mL of Folin Ciocalteau reagent (previously diluted with water 1: 10 v/v) and 800 µL of 0.7 M sodium carbonate for 30 min at room temperature for colour development. The absorbance of the reaction mixture was then measured at 765 nm using Advance UV/VIS Model SE 807 Spectrophotometer (India) and all measurements were taken in triplicate.

Determination of total flavonoid content

The total flavonoid content of the plant extracts was determined using the method described by Ordon Ez *et al.*[17] A volume of 500 µL (240 µg/mL) of each sample was mixed with 500 µL of methanol, 50 µL of 10% aluminum chloride, 50 µL of 1 mol/L potassium acetate and 1.4 mL distilled water. After one hour at room temperature, the absorbance of the reaction mixture was measured in triplicate at 420 nm using Advance UV/VIS Model SE 807 Spectrophotometer (India).

Assay of ferric reducing antioxidant power

The ferric reducing potential of the extracts was assayed as described by Oyaizu[18] with slight modifications. Volume of 1 mL of each extracts at different concentrations (30, 60, 120 and 240 µg/mL) were incubated with 1 mL each of 0.2 M sodium phosphate buffer at pH 6.6 and 1% potassium ferricyanide. After 30 min of incubation at 50°C, the reaction mixture was acidified with1 mL of 10% trichloroacetic acid after which 1 mL of the acidified sample was mixed with 1 mL distilled water and 200 µL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm using Advance UV/VIS Model SE 807 Spectrophotometer (India). The reducing power of the extracts was compared with ascorbic acid.

Assay of α-amylase inhibitory effect

The inhibition of α-amylase can be measured *in vitro* by hydrolysis of starch in the presence of α-amylase enzyme. The α-amylase inhibitory effect of the plant extracts was carried out according to the modified method of McCue and Shetty.[19] Briefly, 250 µL of each extract at different concentrations (30, 60, 120 and 240 µg/mL) were measured followed by addition of 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution. The solution was pre- incubated at 25°C for 10 min followed by addition of 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) at time intervals of 10 sec. The resulting solution was further incubated at 25°C for 10 min and the reaction was terminated by adding 1 mL of dinitrosalicyclic acid (DNS) reagent. The reaction mixture was then boiled for 10 min, cooled at room temperature and diluted with 5 mL of distilled water before taking absorbance at 540 nm using Advance UV/VIS Model SE 807 Spectrophotometer (India). Control and standard

were prepared alongside using the same procedure but replacing the extract with distilled water and acarbose respectively. The percentage inhibition was calculated as follows:

$$\text{Percentage inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of Extract}]}{[\text{Absorbance of Control}} \times 100$$

Assay of inhibition of hemoglobin glycosylation

The inhibition of non-enzymatic glycosylation of haemoglobin by different extracts was assayed following the method described by Pal and Dutta.[20] Briefly, 1 mL each of glucose (2%), hemoglobin (0.06%) and gentamycin (0.02%) prepared in 0.01 M phosphate buffer at pH 7.4 were mixed with 1 mL of each extract at different concentrations (30, 60, 120 and 240 µg/mL) which were dissolved in dimethyl sulfoxide (DMSO). The mixture was incubated in the dark at room temperature for 72 h after which absorbance was measured at 520 nm using Advance UV/VIS Model SE 807 Spectrophotometer (India) with gallic acid as the standard. The percentage inhibition was calculated according to the following formula:

$$\text{Percentage inhibition} = \frac{[\text{Absorbance of Control} - \text{Absorbance of Extract}]}{[\text{Absorbance of Control}} \times 100$$

Statistical analysis

Data were analyzed using SPSS/10 with the results expressed as mean ± Standard deviation (SD) for five determinations. Statistical evaluations were assayed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparison. Differences between means were considered statistically significant at $p < 0.05$.

RESULTS

The yields recovered from ethanol, methanol and distilled water extraction of different plant parts are presented in Table 2. The distilled water extract of *C. paradisi* leaf gave the highest yield of 13.80 g followed by distilled water and ethanol extracts of *C. papaya* leaf with 11.65 g and 9.33 g respectively. The lowest recovery of 0.04 g was obtained from methanol extract of *M. indica* stem bark. Generally, methanol extracts gave lower yields while distilled water extraction produced higher recovery when compared with ethanol and methanol.

The concentrations of polyphenol and flavonoid in the solvent extracts of different plant parts are presented in Tables 3 and 4. *A. occidentale* leaf stood out as all the three extracts (ethanol, methanol and distilled water) were characterized by the presence of significant ($p < 0.05$) high concentrations of total polyphenol and flavonoid compared to other solvent extracts of the same or different plant parts. Other plants with significant concentration of polyphenol include *C. papaya* leaf, *F. asperifolia* leaf, *H. sabdariffa* stem bark, *K. senegalensis* stem bark, *O. gratissimum* leaf and *P. biglobosa* stem bark (Table 3). For flavonoid content, we observed that the same set of plants showed significant higher concentration with slight difference in the solvent (Table 4). The data obtained in respect of ferric reducing antioxidant power indicated that the reductive capability of the extracts was concentration dependent with 240 µg/mL giving the best result. This concentration was therefore chosen to assess the antioxidant potential of the plants studied (Table 5). The results showed that solvent

Table 2: Percentage Yield from Solvent Extraction of Different Plant Parts.

	Extract/Yield (%)		
Plant/Part	**Ethanol**	**Methanol**	**Distilled Water**
A. cordifolea Leaf	1.93	8.60	7.17
A. occidentale Leaf	8.45	1.10	3.23
A. djalonensis Leaf	0.93	0.50	5.48
B. ferruginea Leaf	2.90	5.97	7.20
B. ferruginea Seed	2.21	1.50	3.30
B. ferruginea Stem Bark	3.20	3.33	3.80
C. papaya Leaf	9.33	5.25	11.65
C. *pentandra* Stem Bark	1.40	3.33	2.53
C. *paradisi* Leaf	2.45	1.05	13.80
C. ferruginea Leaf	2.25	1.80	5.43
F. asperifolia Leaf	2.50	0.90	3.78
F. exasperata Leaf	0.63	0.70	3.38
G. latifolium Leaf	3.50	4.10	7.76
H. sabdariffa Stem Bark	2.93	1.08	8.60
H. umbellata Seed	2.53	3.10	5.70
I. pulchra Leaf	1.53	0.60	6.20
K. senegalensis Leaf	4.03	3.63	7.40
K. senegalensis Stem Bark	6.78	1.18	6.48
M. indica Leaf	4.03	5.23	5.90
M. indica Stem Bark	7.05	0.40	1.53
M. Lucida Leaf	0.60	0.63	8.10
N. latifolia Leaf	1.85	2.55	1.13
O. gratissimum Leaf	4.18	2.08	6.40
P. biglobosa Stem Bark	4.60	0.80	2.56
V. album Leaf	1.50	2.10	5.48
V. album Seed	3.13	4.50	5.36

Table 3: Total Polyphenol Content (mg/g TAE) in Solvent Extracts of Different Plant Parts.

	Extracts		
Plant/Part	**Ethanol**	**Methanol**	**Distilled Water**
A. cordifolea Leaf	6.42 ± 0.46^{a}	6.10 ± 0.43^{a}	5.51 ± 0.54^{a}
A. occidentale Leaf	53.95 ± 5.12^{a**}	48.07 ± 4.07^{b**}	40.67 ± 3.96^{c**}
A. djalonensis Leaf	1.61 ± 0.14^{a}	1.53 ± 0.16^{a}	2.31 ± 0.14^{b}
B. ferruginea Leaf	4.43±0.08^{a}	3.83±0.04^{a}	5.30 ± 0.08^{b}
B. ferruginea Seed	0.46 ± 0.08^{a}	0.86 ± 0.05^{a}	1.53 ± 0.05^{b}
B. ferruginea Stem Bark	1.88 ± 0.60^{a}	1.72 ± 0.09^{a}	5.03 ± 0.05^{b}
C. papaya Leaf	33.52 ± 1.77^{a}	20.60 ± 2.87^{a}	47.63 ± 3.40^{b**}
C. *pentandra* Stem Bark	6.06 ± 0.05^{a}	10.90 ± 0.09^{b}	3.83 ± 0.08^{c}
C. *paradisi* Leaf	2.15 ± 0.06^{a}	1.71 ± 0.03^{b}	2.03 ± 0.05^{a}
C. ferruginea Leaf	1.47 ± 0.04^{a}	1.30 ± 0.06^{a}	1.87 ± 0.03^{b}
F. asperifolia Leaf	16.61 ± 0.89^{a}	27.82 ± 1.00^{b}	44.58 ± 4.27^{c**}
F. exasperata Leaf	1.23 ± 0.04^{a}	1.24 ± 0.01^{a}	1.22 ± 0.01^{a}
G. latifolium Leaf	1.38 ± 0.02^{a}	1.06 ± 0.02^{b}	1.09 ± 0.04^{b}
H. sabdariffa Stem Bark	24.19 ± 1.22^{a}	12.25 ± 0.76^{b}	40.39 ± 4.20^{c**}
H. umbellata Seed	0.34±0.02^{a}	0.4 5± 0.03^{b}	0.31 ± 0.02^{a}
I. pulchra Leaf	1.77 ± 0.02 ^{a}	2.24 ± 0.04^{b}	1.94 ± 0.05 ^{a}
K. senegalensis Leaf	4.80 ± 0.17^{a}	4.38 ± 0.30^{a}	3.18 ± 0.09^{b}
K. senegalensis Stem Bark	16.56 ± 1.91^{a}	11.20 ± 0.57^{b}	42.40 ± 4.80^{c**}
M. indica Leaf	3.92 ± 0.17^{a}	4.20 ± 0.11^{a}	3.74 ± 0.15^{a}
M. indica Stem Bark	14.36 ± 0.16^{a}	4.74 ± 0.04^{b}	12.22 ± 0.18^{c}
M. Lucida Leaf	0.64 ± 0.06^{a}	0.88 ± 0.06^{a}	0.54 ± 0.05^{a}
N. latifolia Leaf	1.88 ± 0.06^{a}	2.15 ± 0.09^{a}	5.37 ± 0.05^{b}
O. gratissimum Leaf	39.30 ± 5.43^{a**}	11.00 ± 1.02^{b}	42.60 ± 4.06^{c**}
P. biglobosa Stem Bark	42.84 ± 3.64^{a**}	14.45 ± 0.60^{b}	24.12 ± 0.70^{c}
V. album Leaf	3.33 ± 0.08^{a}	3.11 ± 0.02^{a}	1.72 ± 0.05^{b}
V. album Seed	0.91 ± 0.03^{a}	0.74 ± 0.01^{a}	0.41 ± 0.02^{b}

Data are presented as mean ± SD of three replicates at 240 µg/ml. Values with different letters along the same row for each plant part are significantly different ($p < 0.05$), ** indicate highly significant ($p < 0.05$) difference.

extracts of *A. occidentale* leaves demonstrated significant ($p < 0.05$) higher ferric reducing activity when compared to other plants; with the ethanol and distilled water extracts of the plant showing better activity when compared with ascorbic acid. Interestingly, all the plants reported to have high concentrations of polyphenol and flavonoid in this study also exhibited strong ferric reducing power comparable to ascorbic acid (Table 5). Generally, plant parts extracted with distilled water showed better antioxidant activity compared to ethanol and methanol irrespective of the plant parts.

In this study, the inhibition of α-amylase and haemoglobin glycosylation by plant extracts was also best at 240 µg/mL; hence the choice of this concentration to assess these parameters (Tables 6 and 7). It was clearly observed that leaf extracts of *A. occidentale*, *C. papaya*, *F. asperifolia*, *O. gratissimum* as well as stem bark extracts of *H. sabdariffa*, *K. senegalensis* and *P. biglobosa* exhibited significant ($p < 0.05$) higher inhibitory effect which was comparable to acarbose and gallic acid (Tables 6 and 7).

DISCUSSION

The administration of herbal preparations with regards to diabetes treatment has raised concerns due to absence of adequate scientific basis for their efficacy and safety. However, the present study has clearly demonstrated the potential of some of the traditionally employed medicinal plants in Nigeria as antidiabetic agents.

Polyphenols and flavonoids are the major plant components with antioxidant activity and it is likely that the medicinal properties of plant extracts may be due to the redox properties of these compounds. Previous studies have implicated polyphenols and other related com-

Table 4: Total Flavonoid Content (mg/g QE) in Solvent Extracts of Different Plant Parts.

	Extracts		
Plant/Part	**Ethanol**	**Methanol**	**Distilled Water**
A. cordifolea Leaf	0.45 ± 0.06^{a}	0.48 ± 0.04^{a}	0.42 ± 0.04^{a}
A. occidentale Leaf	38.58 ± 4.63^{a**}	35.83 ± 5.02^{b**}	31.08 ± 4.27^{c**}
A. djalonensis Leaf	4.04 ± 0.12^{a}	2.34 ± 0.17^{b}	6.01 ± 0.14^{c}
B. ferruginea Leaf	4.45 ± 0.10^{a}	3.15 ± 0.11^{b}	4.77 ± 0.16^{a}
B. ferruginea Seed	0.14 ± 0.01^{a}	0.14 ± 0.01^{a}	0.30 ± 0.01^{b}
B. ferruginea Stem Bark	3.95 ± 0.15^{a}	3.50 ± 0.11^{a}	6.83 ± 0.63^{b}
C. papaya Leaf	16.43 ± 1.81^{a}	10.00 ± 0.49^{b}	28.83 ± 5.03^{c**}
C. *pentandra* Stem Bark	4.00 ± 0.12^{a}	8.08 ± 0.34^{b}	5.63 ± 0.17^{c}
C. *paradisi* Leaf	4.13 ± 0.07^{a}	3.55 ± 0.10^{b}	4.01 ± 0.13^{a}
C. ferruginea Leaf	5.49 ± 0.12^{a}	5.48 ± 0.10^{a}	6.53 ± 0.10^{b}
F. asperifolia Leaf	11.00 ± 0.86^{a}	22.50 ± 1.59^{b}	32.75 ± 4.06^{c**}
F. exasperata Leaf	0.85 ± 0.02^{a}	0.88 ± 0.06^{a}	0.83 ± 0.01^{a}
G. latifolium Leaf	0.88 ± 0.02^{a}	0.76 ± 0.01^{a}	0.86 ± 0.01^{a}
H. sabdariffa Stem Bark	12.83 ± 0.76^{a}	10.75 ± 0.50^{a}	26.36 ± 3.91^{b**}
H. umbellata Seed	0.33 ± 0.03^{a}	0.41 ± 0.02^{a}	0.26 ± 0.04^{a}
I. pulchra Leaf	0.45 ± 0.01^{a}	0.85 ± 0.03^{b}	0.71 ± 0.04^{b}
K. senegalensis Leaf	1.53 ± 0.12^{a}	1.39 ± 0.08^{a}	1.30 ± 0.14^{a}
K. senegalensis Stem Bark	27.80 ± 4.08^{a**}	13.20 ± 1.04^{b}	31.80 ± 4.04^{c**}
M. indica Leaf	4.53 ± 0.53^{a}	8.16 ± 0.67^{b}	4.12 ± 0.39^{a}
M. indica Stem Bark	30.50 ± 1.33^{a}	12.50 ± 0.58^{b}	21.04 ± 0.67^{c}
M. Lucida Leaf	0.19 ± 0.02^{a}	0.49 ± 0.04^{b}	0.36 ± 0.08^{c}
N. latifolia Leaf	2.07 ± 0.04^{a}	2.87 ± 0.02^{b}	3.84 ± 0.08^{c}
O. gratissimum Leaf	29.69 ± 4.18^{a**}	4.48 ± 0.56^{b}	16.05 ± 2.28^{c}
P. biglobosa Stem Bark	27.50 ± 4.30^{a**}	10.15 ± 0.47^{b}	19.38 ± 1.04^{c}
V. album Leaf	4.77 ± 0.31^{a}	3.98 ± 0.43^{a}	3.86 ± 0.35^{a}
V. album Seed	0.63 ± 0.03^{a}	0.62 ± 0.02^{a}	0.60 ± 0.02^{a}

Data are presented as mean ± SD of three replicates at 240 µg/ml. Values with different letters along the same row for each plant part are significantly different ($p < 0.05$), ** indicate highly significant ($p < 0.05$) difference.

pounds in a number of therapeutic potentials including antioxidant, antidiabetic, anticancer and antimicrobial effects.[21] In fact, most of the medicinal applications of plant-based formulations have been correlated to the polyphenols present in such plant species.[22] In the current study, extracts of seven plants namely *A. occidentale*, *C. papaya*, *F. asperifolia*, *H. sabdariffa*, *K. senegalensis*, *O. gratissimum* and *P. biglobosa* comprising leaf and stem bark possessed considerable concentrations of polyphenol and flavonoid. Earlier reports have shown strong correlations between polyphenol content and antioxidant activity in many medicinal plants.[23,24] Hodzic *et al.*[25] also reported that the concentration of polyphenols has a considerable impact on the antioxidant capacity of plant extracts. These compounds play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.[26] The hydroxyl groups attached to the aromatic ring structure of flavonoid enable them to undergo redox reaction and consequently scavenge free radicals.[27] This is an indication that extracts from the seven plants mentioned earlier possess considerable antioxidant properties. Consistent with this study, the antioxidant and antidiabetic activities of *A. melegueta* was attributed to rich content of flavonoids and other phenolics.[22,28,29] Findings from the present study therefore indicate that polyphenols and flavonoids form major components of *A. occidentale*, *C. papaya*, *F. asperifolia*, *H. sabdariffa*, *K. senegalensis*, *O. gratissimum* and *P. biglobosa*; suggesting that their antioxidant and antidiabetic activities could be attributed to the presence of these valuable compounds.

The reducing power of a compound is related to its electron transfer ability and may serve as an indicator

Table 5: Percentage Ferric Reducing Antioxidant Power of Solvent Extracts of Different Plant Parts Relative to Ascorbic Acid.

	Extracts			
Plant/Part	**Ethanol**	**Methanol**	**Distilled Water**	**Ascorbic acid**
A. cordifolea Leaf	23.73 ± 1.28^{a}	29.43 ± 2.24^{b}	10.35 ± 0.60^{c}	61.60 ± 2.96^{d}
A. occidentale Leaf	74.53 ± 3.32^{a**}	60.41 ± 1.60^{b**}	68.07 ± 1.07^{a**}	61.60 ± 2.96^{b}
A. djalonensis Leaf	12.48 ± 0.78^{a}	8.73 ± 0.93^{b}	13.80 ± 0.66^{a}	61.60 ± 2.96^{c}
B. ferruginea Leaf	18.42 ± 0.16^{a}	16.98 ± 0.80^{a}	26.82 ± 1.03^{b}	61.60 ± 2.96^{c}
B. ferruginea Seed	11.42 ± 0.90^{a}	14.23 ± 0.37^{a}	20.37 ± 2.06^{b}	61.60 ± 2.96^{c}
B. ferruginea Stem Bark	12.43 ± 0.83^{a}	10.95 ± 0.71^{a}	20.71 ± 1.04^{b}	61.60 ± 2.96^{c}
C. papaya Leaf	45.32 ± 2.47^{a}	35.20 ± 1.07^{b}	57.43 ± 2.30^{c**}	61.60 ± 2.96^{c}
C. *pentandra* Stem Bark	24.93 ± 1.93^{a}	38.28 ± 3.33^{b}	10.85 ± 0.67^{c}	61.60 ± 2.96^{d}
C. *paradisi* Leaf	20.35 ± 1.01^{a}	13.53 ± 0.90^{b}	32.72 ± 2.15^{c}	61.60 ± 2.96^{d}
C. ferruginea Leaf	13.87 ± 0.53^{a}	12.63 ± 0.49^{a}	22.15 ± 1.32^{b}	61.60 ± 2.96^{c}
F. asperifolia Leaf	26.51 ± 2.09^{a}	33.82 ± 2.10^{a}	52.88 ± 3.17^{b**}	61.60 ± 2.96^{b}
F. exasperata Leaf	9.25 ± 0.52^{a}	18.38 ± 0.38^{b}	11.53 ± 0.83^{a}	61.60 ± 2.96^{c}
G. latifolium Leaf	21.17 ± 0.66^{a}	13.87 ± 0.14^{b}	13.97 ± 0.80^{b}	61.60 ± 2.96^{c}
H. sabdariffa Stem Bark	30.91 ± 1.92^{a}	20.51 ± 1.12^{b}	54.79 ± 2.01^{c**}	61.60 ± 2.96^{c}
H. umbellata Seed	12.63 ± 0.70^{a}	22.17 ± 1.08^{b}	12.08 ± 0.83^{a}	61.60 ± 2.96^{c}
I. pulchra Leaf	11.68 ± 0.90^{a}	22.88 ± 2.12^{b}	12.27 ± 0.97^{a}	61.60 ± 2.96^{c}
K. senegalensis Leaf	20.93 ± 0.74^{a}	19.68 ± 0.62^{a}	10.08 ± 0.42^{b}	61.60 ± 2.96^{c}
K. senegalensis Stem Bark	37.66 ± 1.01^{a}	20.12 ± 1.12^{b}	63.20 ± 2.90^{c**}	61.60± 2.96^{c}
M. indica Leaf	39.42 ± 1.19^{a}	42.83 ± 0.50^{a}	39.00 ± 1.37^{a}	61.60 ± 2.96^{b}
M. indica Stem Bark	35.14 ± 1.36^{a}	17.37 ± 0.47^{b}	32.12 ± 1.72^{a}	61.60 ± 2.96^{c}
M. Lucida Leaf	11.57 ± 0.43^{a}	19.97 ± 0.36^{b}	10.88 ± 0.57^{a}	61.60 ± 2.96^{c}
N. latifolia Leaf	31.17 ± 1.09^{a}	40.95 ± 1.15^{b}	42.43 ± 1.33^{b}	61.60± 2.96^{c}
O. gratissimum Leaf	57.99 ± 2.30^{a**}	20.40 ± 1.10^{b}	63.96 ± 1.69^{a**}	61.60 ± 2.96^{a}
P. biglobosa Stem Bark	65.42 ± 3.48^{a**}	22.46 ± 1.06^{b}	46.22 ± 4.07^{c}	61.60 ± 2.96^{a}
V. album Leaf	21.02 ± 1.08^{a}	13.27 ± 1.07^{b}	8.78 ± 0.62^{c}	61.60 ± 2.96^{d}
V. album Seed	18.37 ± 0.26^{a}	17.40 ± 0.17^{a}	10.38 ± 0.10^{b}	61.60 ± 2.96^{c}

Data are presented as mean ± SD of three replicates at 240 µg/ml. Values with different letters along the same row for each plant part are significantly different, ** $p < 0.05$ compared favourably with ascorbic acid.

of its potential antioxidant activity.[30] The ferric reducing assay is widely used to evaluate the antioxidant potential of polyphenols.[31] Again, extracts from the seven plants earlier identified demonstrate good ferric reducing ability which indicates their strong antioxidant activity. This property enables herbs to mop up noxious toxic metabolites released during pathological states and confer protection on the affected organs. The possible mechanism by which the extract exerts its effect may be by prevention of chain initiation or decomposition of peroxides.[32] The reductive capability of the extract was concentration dependent which is an indication that antioxidant activity is likely to increase with higher concentrations. Many of the complications of diabetes have been linked to oxidative stress; and antioxidants have been considered as treatments.[33,34] Hence, it could be suggested that antioxidant action may be an important property of plant medicines associated with diabetes. The present study has clearly demonstrated the antioxidant capabilities of *A. occidentale*, *C. papaya*, *F. asperifolia*, *H. sabdariffa*, *K. senegalensis*, *O. gratissimum* and *P. biglobosa*; and their activity is comparable to ascorbic acid, a standard antioxidant.

α-amylase is one of the main enzymes in human body that is responsible for the breakdown of starch to more simple sugars. The enzyme hydrolyzes complex polysaccharides to produce oligosaccharides and disaccharides which are then broken down into monosaccharides by α-glycosidase. The monosaccharides are then absorbed through the small intestine into the hepatic portal vein and increase postprandial glucose levels.[35,36] It has been established that α-amylase inhibitors from natural

Table 6: Percentage α -Amylase Inhibition by Solvent Extracts of Different Plant Parts Relative to Acarbose.

	Extracts			
Plant/Part	**Ethanol**	**Methanol**	**Distilled Water**	**Acarbose**
A. cordifolea Leaf	28.17 ±1.36^{a}	16.01 ±1.67^{b}	13.96 ±1.06^{b}	45.20 ± 4.06^{c}
A. occidentale Leaf	48.35 ±3.02^{a**}	22.10 ±1.08^{b}	40.80 ± 2.97^{a**}	45.20 ± 4.06^{a}
A. djalonensis Leaf	19.58 ±2.73^{a}	20.42 ±2.53^{a}	21.67 ± 2.91^{a}	45.20 ± 4.06^{b}
B. ferruginea Leaf	20.91 ±1.16^{a}	24.89 ±1.81^{a}	20.21 ± 1.32^{a}	45.20 ± 4.06^{b}
B. ferruginea Seed	14.92 ±1.15^{a}	18.83 ±1.08^{a}	29.07 ± 1.04^{b}	45.20 ± 4.06^{c}
B. ferruginea Stem Bark	19.95 ± 1.25^{a}	17.13 ± 1.21^{a}	31.53 ± 1.01^{b}	45.20 ± 4.06^{c}
C. papaya Leaf	21.23 ± 1.07^{a}	11.42 ± 0.78^{b}	40.76 ± 3.20^{c**}	45.20 ± 4.06^{c}
C. *pentandra* Stem Bark	21.62 ± 1.06^{a}	30.49 ± 1.03^{b}	11.89 ± 0.75^{c}	45.20 ± 4.06^{d}
C. *paradisi* Leaf	19.60 ± 1.04^{a}	10.44 ± 0.72^{b}	19.04 ± 0.94^{a}	45.20 ± 4.06^{c}
C. ferruginea Leaf	18.60 ± 0.48^{a}	16.88 ± 1.05^{a}	28.36 ± 1.04^{b}	45.20 ± 4.06^{c}
F. asperifolia Leaf	15.16 ± 0.79^{a}	25.60 ± 1.01^{b}	44.50 ± 2.18^{c**}	45.20 ± 4.06^{c}
F. exasperata Leaf	12.51 ± 0.55^{a}	19.70 ± 0.40^{b}	20.95 ± 0.34^{b}	45.20 ± 4.06^{c}
G. latifolium Leaf	23.47 ± 1.67^{a}	12.18 ± 0.90^{b}	13.76 ± 0.76^{b}	45.20 ± 4.06^{c}
H. sabdariffa Stem Bark	28.25 ± 0.91^{a}	17.11 ± 1.01^{b}	46.79 ± 1.80^{c**}	45.20 ± 4.06^{c}
H. umbellata Seed	22.50 ± 0.58^{a}	31.75 ± 1.04^{b}	28.91 ± 2.01^{b}	45.20 ± 4.06^{c}
I. pulchra Leaf	23.18 ± 0.53^{a}	34.45 ± 0.45^{b}	25.10 ± 0.41^{a}	45.20 ± 4.06^{c}
K. senegalensis Leaf	21.61 ± 1.04^{a}	12.48 ± 0.49^{b}	18.56 ± 0.69^{a}	45.20 ± 4.06^{c}
K. senegalensis Stem Bark	39.66 ± 2.01^{a**}	15.12 ± 1.76^{b}	44.40 ± 3.80^{a**}	45.20 ± 4.06^{a}
M. indica Leaf	12.96 ± 1.06^{a}	22.73 ± 2.01^{b}	18.81 ± 1.07^{b}	45.20 ± 4.06^{c}
M. indica Stem Bark	29.14 ± 1.06^{a}	13.43 ± 1.01^{b}	25.62 ± 1.88^{a}	45.20 ± 4.06^{c}
M. Lucida Leaf	20.81 ±1.62^{a}	23.55 ±1.75^{a}	11.50 ± 0.72^{b}	45.20 ± 4.06^{c}
N. latifolia Leaf	14.99 ±1.04^{a}	24.82 ±1.22^{b}	27.98 ± 2.16^{b}	45.20 ± 4.06^{c}
O. gratissimum Leaf	48.99 ±3.30^{a**}	26.90 ±2.52^{b}	44.90 ± 3.60^{a**}	45.20 ± 4.06^{c}
P. biglobosa Stem Bark	46.45 ±2.14^{a**}	20.22 ±1.81^{b}	49.62 ± 3.17^{a**}	45.20 ± 4.06^{a}
V. album Leaf	11.89 ±1.36^{a}	22.95 ±0.62^{b}	11.32 ± 0.68^{a}	45.20 ± 4.06^{c}
V. album Seed	29.07 ±2.03^{a}	25.25 ±2.45^{a}	11.31 ± 0.82^{b}	45.20 ± 4.06^{c}

Data are presented as mean ± SD of three determinations at 240 µg/ml. Values with different letters along the same row for each plant part are significantly different, ** $p < 0.05$ compared favourably with acarbose.

sources play a significant role in diabetes management and control. This is achieved via a decrease in post-prandial hyperglycemia through inhibition of α-amylase actions.[37] From our findings, extracts of *A. occidentale*, *C. papaya*, *F. asperifolia*, *H. sabdariffa*, *K. senegalensis*, *O. gratissimum* and *P. biglobosa* demonstrated significant α-amylase inhibition, indicating their potential role as an antidiabetic agent. This finding is in agreement with previous studies using other medicinal plants.[22,37] Hence, this study support the case that natural products from medicinal plants have α-amylase inhibitory activity and could be utilized as a successful therapy for the management of postprandial hyperglycemia with minimal adverse effects.

To further explore the antidiabetic potential of the plant extracts, their ability to inhibit glycosylation of hemoglobin was evaluated. Glycosylation refers to the non-enzymatic reaction between reducing sugars and proteins (hemoglobin, albumin) and usually contributes enormously to the formation of advanced glycation end products.[38] Human beings minimize the production of reactive oxygen species by enzymatic and non-enzymatic antioxidant mechanism, which plays a key role in many degenerative diseases including diabetes. High glucose levels in body leads to its binding to hemoglobin which may result in the production of reactive oxygen species. Previous studies have reported that end products of glycosylation can be inhibited by plant extracts.[39] It is evident from our study that the same set of plants as obtained for α-amylase demonstrated significant inhibition of haemoglobin glycosylation which was comparable to gallic acid. This further indicates the potential role of these plants as an antidiabetic agent.

Table 7: Percentage Inhibition of Haemoglobin Glycosylation by Solvent Extracts of Different Plant Parts Relative to Gallic Acid.

	Extracts			
Plant/Part	**Ethanol**	**Methanol**	**Distilled Water**	**Gallic Acid**
A. cordifolea Leaf	37.45 ± 2.57^{a}	22.80 ± 1.50^{b}	20.48 ± 1.08^{b}	63.48 ± 3.75^{c}
A. occidentale Leaf	68.53 ± 3.95^{a**}	34.50 ± 1.29^{b}	65.48 ± 2.47^{a**}	63.48 ± 3.75^{a}
A. djalonensis Leaf	12.60 ± 1^{a}	15.70 ± 1.86^{a}	25.00 ± 1.12^{b}	63.48 ± 3.75^{c}
B. ferruginea Leaf	24.55 ± 1.44^{a}	14.90 ± 1.39^{b}	33.37 ± 2.73^{c}	63.48 ± 3.75^{d}
B. ferruginea Seed	6.14 ± 0.52^{a}	8.74 ± 0.72^{a}	14.39 ± 1.03^{b}	63.48 ± 3.75^{c}
B. ferruginea Stem Bark	14.63 ± 0.89^{a}	13.32 ± 0.80^{a}	25.27 ± 0.58^{b}	63.48 ± 3.75^{c}
C. papaya Leaf	32.33 ± 1.52^{a}	21.41 ± 1.15^{b}	68.47 ± 3.63^{c**}	63.48 ± 3.75^{c}
C. *pentandra* Stem Bark	28.34 ± 0.79^{a}	10.37 ± 0.71^{b}	20.05 ± 0.66^{c}	63.48 ± 3.75^{d}
C. *paradisi* Leaf	23.67 ± 1.80^{a}	20.30 ± 2.95^{a}	36.21 ± 2.17^{b}	63.48 ± 3.75^{c}
C. ferruginea Leaf	9.27 ± 0.65^{a}	7.11 ± 0.96^{a}	18.94 ± 0.91^{b}	63.48 ± 3.75^{c}
F. asperifolia Leaf	24.16 ± 2.61^{a}	37.48 ± 1.28^{b}	68.44 ± 2.58^{c**}	63.48 ± 3.75^{c}
F. exasperata Leaf	7.88 ± 0.33^{a}	8.04 ± 0.49^{a}	17.04 ± 1.07^{b}	63.48 ± 3.75^{c}
G. latifolium Leaf	18.83 ± 1.08^{a}	6.87 ± 0.33^{b}	7.67 ± 0.52^{b}	63.48 ± 3.75^{c}
H. sabdariffa Stem Bark	38.20 ± 1.92^{a}	22.61 ± 2.26^{b}	63.75 ± 2.39^{c}	63.48 ± 3.75^{c}
H. umbellata Seed	16.90 ± 1.35^{a}	8.65 ± 0.69^{b}	16.50 ± 1.82^{a}	63.48 ± 3.75^{c}
I. pulchra Leaf	26.72 ± 1.30^{a}	15.97 ± 1.04^{b}	13.48 ± 0.82^{b}	63.48 ± 3.75^{c}
K. senegalensis Leaf	28.95 ± 1.62^{a}	26.05 ± 1.36^{a}	14.17 ± 0.85^{b}	63.48 ± 3.75^{c}
K. senegalensis Stem Bark	59.66 ± 2.01^{a**}	23.08 ± 1.71^{b}	61.00 ± 2.80^{a**}	63.48 ± 3.75^{a}
M. indica Leaf	23.90 ± 1.52^{a}	37.54 ± 1.13^{b}	21.04 ± 1.16^{a}	63.48 ± 3.75^{c}
M. indica Stem Bark	36.14 ± 2.36^{a}	23.77 ± 1.74^{b}	40.12 ± 3.88^{a}	63.48 ± 3.75^{c}
M. Lucida Leaf	8.66 ± 0.49^{a}	10.95 ± 0.86^{a}	19.46 ± 0.46^{b}	63.48 ± 3.75^{c}
N. latifolia Leaf	13.55 ± 1.09^{a}	20.78 ± 0.92^{b}	36.59 ± 1.68^{c}	63.48 ± 3.75^{d}
O. gratissimum Leaf	62.14 ± 2.36^{a**}	40.74 ± 1.45^{b}	58.12 ± 2.88^{a**}	63.48 ± 3.75^{a}
P. biglobosa Stem Bark	65.42 ± 2.84^{a**}	36.46 ± 1.50^{b}	59.24 ± 2.17^{a**}	63.48 ± 3.75^{a}
V. album Leaf	19.54 ± 1.09^{a}	18.92 ± 0.73^{a}	10.12 ± 0.35^{b}	63.48 ± 3.75^{c}
V. album Seed	11.45 ± 0.63^{a}	9.25 ± 0.48^{a}	5.51 ± 0.35^{b}	63.48 ± 3.75^{c}

Data are presented as mean ± SD of three determinations at 240 µg/ml. Values with different letters along the same row for each plant part are significantly different, ** $p < 0.05$ compared favourably with gallic acid.

CONCLUSION

Our findings revealed a positive correlation between the polyphenol/flavonoid concentration and *in vitro* antidiabetic/antioxidant activities of plant extracts. Hence, these *in vitro* assays indicated that plants extracts may be a significant source of natural antioxidant and antidiabetic agents. However, out of the 21 medicinal plants examined in this study, which are used in Nigerian traditional medicine for the treatment of diabetes, only seven actually demonstrated antidiabetic activity based on the scientific indices employed. This does not suggest that the others cannot find medicinal usefulness but further implies that more scientific investigations should be carried out in order to validate the antidiabetic activity of medicinal plants as claimed by traditional healers. Finally, our findings have justified and offered scientific credence to the folkloric use of some of the herbs in Nigerian as antidiabetic agents.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

IDDM: Insulin–dependent diabetes mellitus; **NIDDM:** Non-insulin–dependent diabetes mellitus; **DNS:** Dinitrosalicyclic acid; **DMSO:** Dimethyl sulfoxide.

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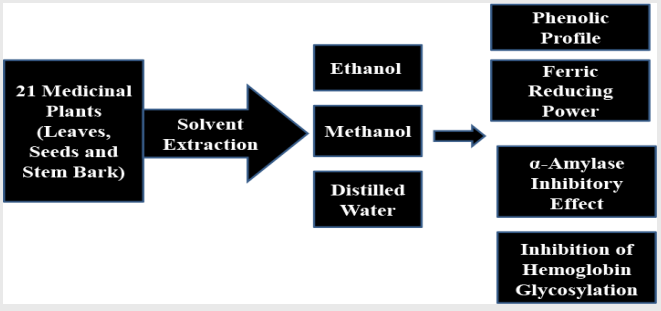
PICTORIAL ABSTRACT



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Professor Francis Lewu, obtained BSc (Agriculture) from the University of Agriculture Abeokuta and MSc Agronomy (Soil Microbiology) in the University of Ibadan, Nigeria. He started his career as a Genebank Research Assistant in the International Institute of Tropical Agriculture, IITA, Nigeria; and later proceeded to study PhD in Botany with specialization in plant genetic conservation and propagation at the University of Fort Hare, South Africa. Lewu concentrates his research effort on the autecology and propagation of medicinals and alternative cultivation protocols for field crops and horticultural species. His latest research interest is focused on the influence of nanomaterials on efficacy of medicinal species and their ameliorating effects on biochemicals of screened materials through different propagation and screening methods.

SUMMARY

- The present study investigated the *in vitro* antidiabetic activity of 21 commonly used medicinal plants used for the treatment of diabetes by traditional healers in Nigeria.
- Solvents extracts of 7 plants namely *A. occidentale, C. papaya, F. asperifolia, H. sabdariffa, K. senegalensis, O. gratissimum and P. biglobosa* had considerable concentrations of polyphenolic compounds and demonstrated significant antioxidant/antidiabetic activity.
- Generally, plant parts extracted with distilled water showed better antidiabetic activity compared to ethanol and methanol.
- Overall, findings from this study indicate that plants extracts may be a significant source of natural antioxidant and antidiabetic agents.

Professor Taofik Olatude Sunmonu graduated from the University of Ilorin, Ilorin, Nigeria in 1998 with a B.Sc. degree in Biochemistry. He obtained his M.Sc. and Ph.D. degrees (Biochemistry) from the same University in 2002 and 2006 respectively. He was awarded postdoctoral fellowship and training at Research Centre for Phytomedicine, University of Fort Hare, Alice, South Africa (2009), Alpha Care Centre, Worcestershire, Birmingham, United Kingdom (2011) and Research Centre for Plant Growth and Development, University of KwaZulu-Natal, Pietermaritzburg, South Africa (2013). He was also offered the position of a Visiting Researcher at Cape Peninsula University of Technology, Wellington, South Africa (2018). He has to his credit over 50 in-depth scientific articles, which are published in reputable journals in the areas of Environmental Toxicology, Phytomedicine and Plant Biochemistry.

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