

Characterization and Evaluation of *Vernonia amygdalina* Extracts for its Antidiabetic Potentials

Adewole E^{1,3}, Ojo A¹, Ogunmodede O.T¹, Adewumi D.F¹,
Omoaghe A.O², Jamshed I³

¹Department of Chemical Sciences, Afe Babalola University Ado, Ekiti State, Nigeria

²Department of Physiology, Afe Babalola University Ado, Ekiti State, Nigeria

³Department of Pharmacy, Centre for Advanced Drug Research (CADR), COMSATS Institute of Information Technology, Abbottabad, Pakistan

Abstract: Background: There is increase in the demand for the use of medicinal plants for the treatment of various ailments such as cancer, hypertension and diabetes locally as a result of the inability of the synthesized drugs to completely eradicate the ailments and also with the attendant side effects attached to the use of drugs. **Objective:** The aim of this research work was to investigate the Aldose reductase, α -glucosidase, β -glucosidase and maltase glucoamylase inhibitory potential of the crude extracts of *Vernonia amygdalina*, and characterizing the extracts. **Materials and method:** The leaves of the plant was obtained, air dried and turned to powdered form using commercial grinding machine with dimension 34×38×75. The extracted crudes were subjected into in-vitro studies of the inhibitory potentials using recommended protocols. The IC₅₀ values were calculated using Graph pad prism 5.0 software and the identified compounds were screened for drug properties using ONLINE OSIRIS server explorer. **Results:** The (IC₅₀ 0.6 ± 0.03 µg/mL) of the chloroform extract was better than the methanolic extract of IC₅₀ (1.532±0.63µg/mL) and the results were better than the acarbose standard (IC₅₀ 234.6 ± 2.01µM). The maltase glucoamylase inhibitory potentials of the chloroform and methanolic extracts in the range of IC₅₀ 1.112 ± 0.90 µg/mL and 1.315 ± 0.7 µg/mL. The β -glucosidase screening of the extracts results indicated that they do not have good selective inhibitor properties. IC₅₀ of Aldose reductase (ALR2) of chloroform extract IC₅₀ (1.339±0.264µg/mL) was better than that of methanolic extract of IC₅₀ (1.437±0.6µg/mL) and these values were better when compared with the standard, sorbinil of IC₅₀ (3.10 ±0.20µM).The aldehyde reductase (ALR1) of methanolic extract IC₅₀ (0.325±0.02µg/mL) was better than that of chloroform extract of IC₅₀ (0.964±0.16µg/mL) and when compared with standard 10mM vaproic acid IC₅₀ (57.4 ±10µM).The GC-MS of the chloroform extract revealed compounds which were screened computationally to for various drug properties such as drug likeness, cLogS, cLogP, H-bond acceptor and H-bond Donor. **Conclusion:** The promising inhibitory potential of the plant extracts and characterization of the extract is an indication of the usefulness of the plant for the treatment of diabetes.

Keyword: Diabetes mellitus, drug properties, aldose reductase, α -glucosidase, maltase glucoamylase, β -glucosidase

Introduction

There are widely research studies on the use of medicinal plants for the treatment of diabetes and many studies have revealed the efficacies of crude plant extracts as well as their bioactive compounds in reducing blood glucose levels as reported by Kaushik [1]. It has been reported that decreasing the post prandial glucose level by slowing down the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes (α - and β -glucosidases) in digestive tract is one of the therapeutic approaches to

treat diabetes [2]. There are many synthetic anti-diabetic drugs being used for the management of the ailment however, it has not been reported that novel drugs have been found to completely eradicate the disease [3] and this has justified the continuously searching for the plants with good potent inhibitors of α -glucosidase, β -glucosidase and maltase glucoamylase with no side effect as being characterized with synthetic drugs available. α -glucosidase inhibitors are defined as oral antidiabetic drugs that are widely used for type 2 diabetes

This article is published under the terms of the Creative Commons Attribution License 4.0

Author(s) retain the copyright of this article. Publication rights with Alkhaer Publications.

Published at: <http://www.ijsciences.com/pub/issue/2018-01/>

DOI: 10.18483/ijSci.1462; Online ISSN: 2305-3925; Print ISSN: 2410-4477



Adewole Ezekiel (Correspondence)



adewolen50@yahoo.com



+

mellitus and function primarily by disallowing the digestion of carbohydrates and thus reduces the impact on the blood sugar. There are widely use of manufactured α -glucosidase inhibitors this includes acarbose, miglitol and voglibose. It is has been proofed that α -glucosidase inhibitors are used to establish higher glycemic control over hyperglycemia in DM type 2 patients[4]. Moreover, it is has been reported that Maltase glucoamylase, is an α -glucosidase digestive enzyme and comprises two subunits with different substrate precision[4]. Research has revealed that the recombinant enzyme studies had shown that its N-terminal catalytic domain has highest activity against maltose, while the C-terminal domain has a broader substrate precision and activity against glucose oligomers [5]. It has been earlier reported that Aldose reductase having two isoforms (ALR1 and ALR2) has been implicated in the cause of diabetic problems that may be connected to a much significance flux of glucose through the polyol pathway, caused in tissues such as lens, kidney, retina and nerves at high blood glucose levels. Therefore, the inhibitory property of aldose reductase is gaining recognition as a major therapeutic tool for the treatment of hyperglycemia-induced cardiovascular pathologies [6]. Long term drawbacks of diabetes include among others; cataractogenesis and microangiopathy (including nephropathy, retinopathy and neuropathy) thought to be linked to excess free glucose in corresponding tissues[7]. Widely research work has provided evidence of aldose reductase (AR) implication in diabetes severalty [8, 9]. Scientific investigation to provide inhibitors of AR has been a major task for the chemists in order to effectively treat various degrees of complications associated with diabetes.

V. Amygdalina is one of the widely consumed leaf vegetables in Nigeria and well known as a medicinal plant with many pharmacological uses such as diabetes, fever reduction and recently a non pharmaceutical solution to consistent headache and joint pains connected to AIDS ((an infusion of the plant is taken as needed) [10, 11]. It has been scientifically documented that the roots of the plant has been used as toothache and gingivitis as a result of its proven antimicrobial activity [12]. *V. amygdalina* plant has been researched upon and the experimental evidence for its use in diabetes management as reported by Udoamaka [13] included; 80 mg/kg aqueous extract of the leaves produced a dose dependent decrease in blood glucose levels in normal and alloxan-induced diabetic rabbits [14]; chronic intake of 400mg/kg ethanolic extract of the fresh leaves significantly decreased fasting blood glucose levels, increased serum and pancreatic insulin levels, increased the activity of liver antioxidant enzymes as well as increased the expression and distribution of Glut 4 receptors in

STZ-induced diabetic rats [15]; 150ml of a (1:1:1) decoction mix of the leaves of *Vernonia amygdalina*, *Ocimum gratissimum* and *Gongronema latifolium* decreased baseline blood glucose levels in normal subjects when preadministered to normal subjects 45 minutes before an OGTT [16], however the selected plant has not been evaluated simultaneously for its potent inhibition of α - and β - glucosidases, maltase glucoamylase, aldose reductase (ALR1 and ALR2) and identifying the bioactive compounds of the chloroform crude extract.

Methodology

Research Laboratory

The research work was carried out at the centre for the Advanced Drug Research (CADR), Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan in the month August, 2017.

Materials and Instruments

All the reagents, solvents used are of analytical grade, α -glucosidase (from *Saccharomyces cerevisiae*), substrate p-nitrophenyl α -D-glucopyranoside (pNPG), β -glucosidase (from sweet almonds) and 96 well plates were purchased from Sigma Aldrich.

Plants source

Vernonia amygdalina leaves were collected from farm and were authenticated at the Department of Agricultural Science, Afe Babalola University Ado Ekiti, Nigeria.

Crude extracts preparation

The extraction was done by soaking two hundred grams of powdered samples in 1000ml of chloroform and methanol for a period of five days and filtered through whatman filter paper. The extracts were concentrated using a rotary evaporator at 35°C and the dried extract was stored at room temperature for further use. The stock sample was prepared by dissolving 10mg of dried crude extracts in 1mL of 100% dimethyl sulfoxide (DMSO) and labelled as stock (10mg/ml), working solution was made as 1 mg/ml.

Extraction of Aldose Reductase Enzymes

Material and Instruments used for the extraction procedure

All the chemicals needed for the enzyme extraction were of analytical grade. Substrates (D,L-glyceraldehyde and sodium-D-glucuronate) and nicotinamide adenine dinucleotide phosphate (NADPH) as co-factor were purchased from Sigma Aldrich. Eliza microplate reader was used with a UV range of 340nm for the enzymatic reaction.

Isolation and purification of Aldose reductase enzyme (ALR2)

This was done according to Hayman [17] with minor modification; the modification to the method was done by adding different mass of ammonium sulphate to the supernatant depending on the volume of supernatant collected as percentage of saturation increases.

Isolation and purification of ALR1 (Aldehyde reductase)

Kidneys were removed from the calf soon after slaughtering and dissolved in 3 volumes of 10mM sodium phosphate buffer, pH 7.2, containing 0.25M sucrose, 2.0mM EDTA dipotassium salt and 2.5 mM β-mercaptoethanol. The homogenate was Centrifuged the at 12,000×g at 0-4°C for 30 minutes. The precipitate was discarded as it contained insoluble lipids. Collected supernatant layer was subjected to 40% ammonium sulphate saturation to isolate ALR1. This 40% saturated liquid was centrifuged at 12,000×g at 0-4°C for 30 minutes. Again, precipitate was discarded and supernatant was subjected to 50% saturation with ammonium sulphate salt. The same procedure was repeated with this and in the last step; ammonium sulphate was added to increase saturation up to 75%. Centrifuge the supernatant at 12,000×g at

0-4°C for 30 minutes, causing the precipitation of ALR1. Now precipitated material was collected and supernatant discarded. The precipitated material containing enzyme was re-dissolved in 10mM sodium phosphate buffer, pH 7.2 containing 2.0mMEDTA dipotassium salt and 2.0mM β-mercaptoethanol and dialyzed over night using the same buffer. The dialyzed material containing ALR1 enzyme was stored at -80°C before use [18].

Total Protein Evaluation in the Extracted Enzymes

Test Procedure using total protein kit (Laboratory kit made by: (Chemelex, S.A. Pol.ind.Can, Castells. Industrial 113 NAu J.08420, Canovelles, Barcelona Spain)

- (i) Assay Conditions
 - Wavelength -----540nm(530-550)nm
 - Cuvette-----1cm light path
 - Temperature-----37°C
- (ii) The instrument was adjusted to zero with distilled water
- (iii) Pipette into a cuvette

Table 1: Procedure for the determination of protein content in isolated ALR1 and ALR2 enzymes.

	Blank	Calibrator	Sample
R1 ^a (mL)	0.25	0.25	0.25
Calibrator ^b (μL)	-	6.25	-
Sample(μL)	--	----	6.25

^aTotal protein solution (Ref No: 30350-125ml)

^bTotal protein calibrator (AP: 0314, Ref No 30941-5ml)

(iv) The mixture was done and incubate for 5 minutes at 37°C

(v) Absorbance reading was taken and colour change was stable for 30 minutes.

Calculation: Total Protein = $\frac{A_{SAMPLE}}{A_{standard}} \times 5(calibrator)$

Determination of aldose reductase (ALR2 and ALR1) inhibitory activities

UV spectrophotometer was used at 340 nm in order to determine the activity of aldose reductase by measuring the NADPH consumption. Each well of the 96-well plate contains exactly 100 μL of assay mixture containing phosphate buffer 100 mM at pH 6.2 (10μL), with 10μL of 1mg/ml of crude extracts followed by addition of 35 μL of enzyme and 20 μL of substrate (D,L-glyceraldehyde for ALR2 and sodium-D-glucuronate for ALR1). The mixture was incubated at 37°C for 5 min and for the enzymatic reaction to run properly 0.5mM NADPH (20 μL) as a cofactor was added and reading was taken at 340 nm. The mixture was incubated again at 37 °C for 10 min

and reading was taken at the respective UV range in ELIZA plate reader. For ALR2 (10 mM Sorbini) and ALR1 (vapric acid) of 10 μL each was used as positive control and 20μL buffer solution as negative control respectively.

The enzymatic reaction was run in triplicate with a final volume of 100μL in each well. Absorbance was noted and results were analysed.

α-glucosidase inhibition study

The inhibitory effect of the 1mg/ml of the working solution was performed according to a previously published method by Gao [19].

β -glucosidase inhibition study

The evaluation of inhibitory activity against β -glucosidase was performed following the previously published method by Pérez [20].

Extraction of intestinal maltase-glucoamylase enzyme

Intestinal maltase-glucoamylase was extracted by following the literature reported procedure [21, 22]. The enzyme was extracted from white male rats (1-2 months) weighing 150-250 g and starved for 12 hours before death. Rats were killed by cervical dislocation. Whole intestines were gently removed and washed with ice cold 0.9% NaCl. For extraction, intestines were cleaned and cut longitudinally, and mucosal scrapings of 5-6 rats were combined and homogenized in 50 volumes of 5 mM Ethylenediaminetetraacetic Acid (EDTA); pH 7.0. All the steps were carried out at 4 °C. Homogenized intestines were centrifuged at 15,000 rpm for 45 min at 4 °C and the supernatant was discarded. Pellet was re-suspended in 90 mL of ice-cold water followed by 5 mL of 0.1M-EDTA/ 0.2 M potassium phosphate, pH 7, and 5 mL of 0.1 M cysteine was added. Mixture was incubated at 37 °C for 30 min. Pellet was collected by centrifugation at 15,000 rpm for 45 min and the supernatant was discarded. Intestinal suspension was re-dissolved in 10 mM potassium phosphate buffer, pH 6.8, to which 4 mg papain and 0.4 mg cysteine were added. The activated papain solution was immediately used. Mixture was incubated at 37 °C for 40 min with continuous shaking. It was then centrifuged at 15,000 rpm for 90 min. Supernatant was collected and precipitated with ammonium sulphate to 80% saturation. It was dialyzed overnight against distilled water with three changes of water (40 vol every time). The residue was re-dissolved in 4 mL of 10 mM-potassium phosphate (pH 7) and protein concentration was determined. The extracted enzyme was stored at -80 °C until further use.

Maltase-glucoamylase inhibition assay

Maltase-glucoamylase inhibition assay was carried out using the substrate p-nitrophenyl α -D-glucoside using reported procedure by Tanaka [23]. The activity of the test compounds against maltase glucoamylase was determined by measuring p-nitrophenol at a wavelength of 405 nm. Acarbose was used as a positive control.

Statistical Analysis

The total percentage inhibitions were calculated by the formula:

$\%Inhibition =$

$$\left[100 - \left(\frac{\text{absorbance}_{\text{testwell}}}{\text{absorbance}_{\text{control}}}\right)\right] \times 100 \quad [19]$$

IC₅₀ \geq 50% values of potent inhibitors were determined by testing the serial dilutions of inhibitors and were calculated by using the program PRISM 5.0 (GraphPad, San Diego, California, USA).

GC/MS analysis

GC-MS analysis of methanol and chloroform extracts of were performed using TurboMass GC System, fitted with an Elite-5 capillary column (30 m, 0.25 mm inner diameter, 0.25 μ m film thickness; maximum temperature, 350°C coupled to a Perkin Elmer Clarus 600C MS. Helium was used as gas carrier at a constant flow rate of 1.0 ml/min. The injection, transfer line and ion source temperatures were 280°C. The ionizing energy was 70 eV. The oven temperature was programmed from 70 °C (hold for 2 min) to 280°C (hold for 10 min) at a rate of 5°C/min. The crude extract was solubilised with chloroform and filtered with syringe filter (Corning, 0.45 μ m). Volumes of 1 μ l of the crude extracts were injected with a split ratio 1:20. The data were obtained by collecting the mass spectra within the scan range 50-550 m/z. The identification of chemical compounds in the extracts were based on GC retention time; the mass spectra matched those of standards available at NIST library.

Results

Table 2: Showing the amount of protein content in aldose reductase enzymes

Enzyme	protein content (μ g/ μ L)
ALR1	22.88
ALR2	26.00

Table 3: Showing the results of inhibitory effect of crude extracts

Extracts	α -glucosidase	Maltase glucoamylase	β -glucosidase	ALR1	ALR2
	IC ₅₀ (μ g/mL)	IC ₅₀ (μ g/mL)	% inhibition	IC ₅₀ (μ g/mL)	IC ₅₀ (μ g/mL)
Methanolic extract	1.532 \pm 0.63	1.112 \pm 0.90	12.89 \pm 8.21	0.325 \pm 0.02	1.437 \pm 0.6
chloroform extract	0.600 \pm 0.03	1.315 \pm 0.70	15.58 \pm 4.0	0.964 \pm 0.16	1.339 \pm 0.264
Acarbose ^a	234.6 \pm 2.01(μ M)	234.6 \pm 2.01(μ M)	Not Tested	Not Tested	Not Tested
Castanospermine ^b	Not tested	Not tested	59.98%	Not tested	Not tested
Vaproic acid ^c	Not tested	Not tested	Not tested	57.4 \pm 10	Not tested
Sorbinil ^d	Not tested	Not tested	Not tested	Not tested	3.10 \pm 0.20

 SEM \pm standard mean error

^a α -glucosidase standard

^b β -glucosidase standard

^c ALR1 standard

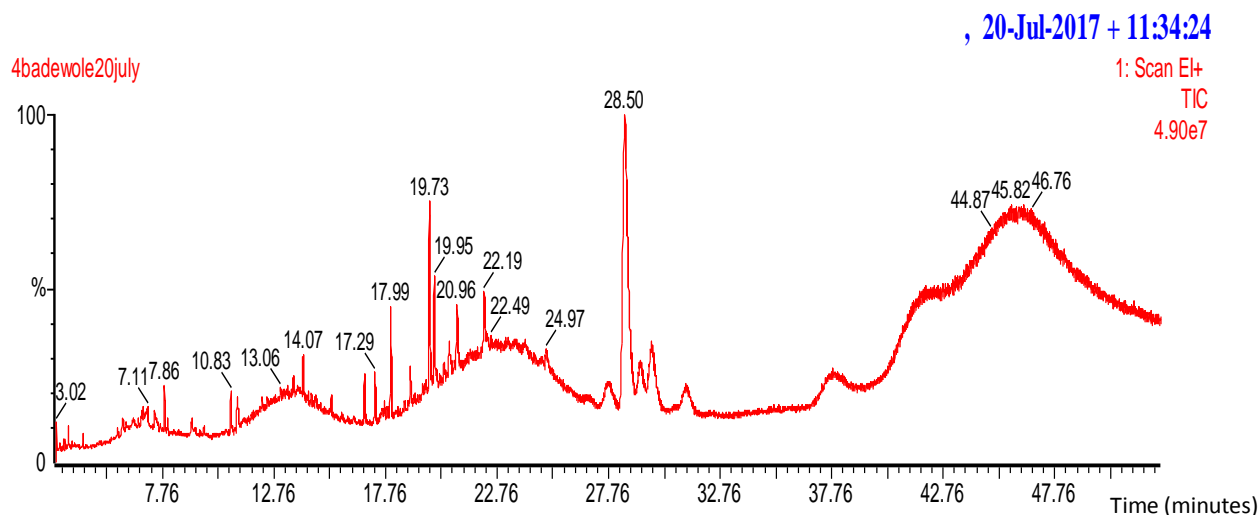
^d ALR2 standard (^dReported IC₅₀ of 3.42 μ M of Sorbinil by Rakowitz *et al.* 24^a and Ali *et. al.*24^b)

 Figure 1: Showing the chromatogram of chloroform crude extract of *V. Amygdalina*

Table 4. Some identified compounds in the Chloroform crude extract of *V.Amygdalina*

Compound name	Molecular formula	Molecular weight	CAS No	Retention time(minutes)
Phytol	C ₂₀ H ₄₀ O	296	150-86-7	13.983
α -cubebene	C ₁₅ H ₂₄	204	17699-14-8	16.839
Oxalic acid	C ₂₁ H ₃₈ O ₄	354	900309-70-5	24.357
3-keto-isosteviol	C ₂₀ H ₂₈ O ₄	332	900255-38-4	19.725
2-Naphthol,1,2,3,4,4A,5,6,7-octahydro-4A-methyl	C ₁₁ H ₁₈ O	166	91253-94-0	35.213
L-(+)- ascorbic acid, 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	652	28474-90-0	39.513
Linolenic acid, trimethyl silyl ester	C ₂₁ H ₃₈ O ₂ Si	350	900141-48-0	49.043
(Z,Z)- α -Farnesene	C ₁₅ H ₂₄	204	900293-03-1	17.975
Oxirane hexadecyl	C ₁₈ H ₃₆ O	268	7390-81-0	29.329

Table 5: Drug properties of identified compounds in the crude extracts determined by OSIRIS property explorer

Compound	Drug likeness	Mutagenic	Tumorigenic	cLog S	cLog P	Polar surface area (Å ²)	%Absorption	H-bond Acceptor	H-bond Donor	Reproductivity	Sp ³ atoms	Aromatic ring	
Phytol	-3.7661	none	None	-	4.633	7.4212	20.23	102.02	1	1	None	19	0
Oxalic acid	-6.1289	high	None	0.066	-1.5754	74.6	83.263	4	2	High	2	0	
Steviol ^a	-5.4797	none	None	-	4.067	3.2941	57.53	89.15215	3	2	None	19	0
2-Naphthol ^b	-2.12	high	High	-	2.926	2.5083	40.46	05.0413	1	1	None	2	4
Ascorbic acid (molecular aspects)	0.023806	None	None	-	0.349	-2.4646	107.22	72.0091	6	4	None	8	0
Farnesene	-7.3918	None	None	-	3.311	6.3983	0	109	0	0	None	7	0
dioxirane ^c	-3.43	none	High	-	1.181	0.4952	25.06	100.3543	2	0	None	3	0

^a (share the same functional group with 3 -keto-isosteviol)^b (share the same functional group 2-Naphthol,1,2,3,4,4A,5,6,7-octahydro-4A-methyl)^c (dioxirane share the functional group with Oxirane hexadecyl)

Discussion

The result from the of table 3, it is shown that the IC_{50} of methanolic extract ($1.53 \pm 0.63 \mu\text{g/mL}$) showed less inhibitory potential against α -glucosidase than that of chloroform extract IC_{50} ($0.600 \pm 0.03 \mu\text{g/mL}$). However, the two results were better than the acarbose standard ($234.6 \pm 2.01 \mu\text{M}$) against α -glucosidase. The IC_{50} both for the extracts of *Vernonia amygdalina* against α -glucosidase were better than the ethanolic extracts of *Andrographis paniculata* and *andrographolide* as reported by Rammohan [25] when compared. The extract of *A. Paniculata* showed α -glucosidase inhibitory effect in a concentration-dependent manner (IC_{50} of $17.2 \pm 0.15 \text{ mg/mL}$) and *andrographolide* demonstrated a similar (IC_{50} of $11.0 \pm 0.28 \text{ mg/mL}$) against α -glucosidase. Furthermore, the α -glucosidase of the *Vernonia amygdalina* extracts were better than the methanolic extracts of *Artocarpus attilis* (IC_{50} $129.85 \pm 10.29 \mu\text{g/mL}$), *A.heterophyllus* (IC_{50} $76.90 \pm 9.55 \mu\text{g/mL}$), *Cinnamomus zeylanicum* (IC_{50} $140.01 \pm 0.08 \mu\text{g/mL}$) and *piper betel* (IC_{50} $96.56 \pm 12.93 \mu\text{g/mL}$) at concentrations ranged from 20 to 100 $\mu\text{g/mL}$ as reported by Sindhu [26]. In addition, the methanolic extract of *B.micrantha* (IC_{50} $1.06 \pm 0.1 \mu\text{g/mL}$) showed good potent and selective inhibitor than *A.calamus* (IC_{50} 1.26 mg/mL) and *N.sativa* (IC_{50} 1.53 mg/mL) as reported by Balaji [27] against α -glucosidase. The IC_{50} ($1.112 \pm 0.90 \mu\text{g/mL}$) of methanolic extract of *Vernonia amygdalina* was better than the chloroform extract of IC_{50} ($1.315 \pm 0.700 \mu\text{g/mL}$) when tested against maltase glucoamylase and the results were better than the standard acarbose (IC_{50} $234.6 \pm 2.01 \mu\text{M}$). However, the IC_{50} both for the extracts of *V.amygdalina* against maltase glucoamylase showed good inhibitory potentials than the methanolic extracts of *Artocarpus attilis* (IC_{50} $118.88 \pm 11.14 \mu\text{g/mL}$), *A.heterophyllus* (IC_{50} $70.58 \pm 9.66 \mu\text{g/mL}$), *Cinnamomus zeylanicum* (IC_{50} $130.55 \pm 10.5 \mu\text{g/mL}$) and *piper betel* (IC_{50} $84.63 \pm 13.09 \mu\text{g/mL}$) at concentrations ranged from 20 to 100 $\mu\text{g/mL}$ as reported by Sindhu [26]. The β -glucosidase screening of the *Vernonia amygdalina* extracts showed that they do not potent and selective inhibition, the methanolic extract had $12.89 \pm 8.21\%$ and chloroform extract had $15.58 \pm 4.00\%$ inhibitory potential against β -glucosidase and these values were less than the Castonospermine standard (59.98%) as reported by Verma [28]. IC_{50} of Aldose reductase (ALR2) of chloroform extract IC_{50} ($1.339 \pm 0.264 \mu\text{g/mL}$) was better than that of methanolic extract of IC_{50} ($1.437 \pm 0.6 \mu\text{g/mL}$) and these values were better when compared with the standard, sorbinil of IC_{50} ($3.10 \pm 0.20 \mu\text{M}$). The aldehyde reductase (ALR1) of methanolic extract IC_{50} ($0.325 \pm 0.02 \mu\text{g/mL}$) was better than that of chloroform extract of IC_{50} ($0.964 \pm 0.16 \mu\text{g/mL}$) and when compared with standard 10mM vaproic acid IC_{50} ($57.4 \pm 10 \mu\text{M}$).

Osiris Drug Properties

Some of the identified compounds were found to have various drug characteristics when screened using OSIRIS Online server explorer [29] and this include; drug likeness, cLogS, cLogP, mutagenic, tumorigenic, irritability, H-bond acceptor and H-bond Donor. The promising inhibitory effect of the plant extracts against aldose reductase, aldehyde reductase, α -glucosidase, glucoamylase and the identified bioactive compounds could be taken as a tool for further insight into the usefulness of the plant for the treatment of diabetes mellitus.

Conclusion

This study discovered the promising and excellent antidiabetic potential of methanolic and chloroform extracts of *V. amygdalina* that can be beneficial for the treatment of diabetes mellitus. This study will help the researcher to uncover the critical areas of using the various drug properties as revealed as a tool for gaining more insight onto the isolation of compounds in the plant with therapeutic properties that many researchers were not able to explore. Thus a new theory on antidiabetic agent may be arrived at.

Acknowledgement

This research work was founded by the CIIT-TWAS postdoctoral fellowship grant to Dr. Adewole in 2017 at Centre for Advanced Drug Research (CADR), COMSATS, Institute of Information Technology, Pakistan.

References

1. G. Kaushik, S. Satya, R. K. Khandelwal and S. N. Naik (2010); commonly consumed Indian plant food materials in the management of diabetes mellitus, *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. Volume 4, pg 21-40.
2. S. Cheplick, Y. I. Kwon, P. Bhowmik and K. Shetty (2010). Phenolic-linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension, *Bioresour. Technol.* 101, 404-413.
3. W.L. Li, H.C. Zheng, J. Bukuru, N. De Kimpe (2004). 'Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus' *Journal of Ethnopharmacology* 92 pg 1-21 (a review)
4. www.Wikipedia.org// α -glucosidase inhibitor
5. Quezada-Calvillo R, Sim L, Ao Z, Hamaker BR, Quaroni A, Brayer GD, Sterchi EE, Robayo-Torres CC, Rose DR, Nichols BL (2008). "Luminal starch substrate "brake" on maltase-glucoamylase activity is located within the glucoamylase subunit". *J. Nutr.* 138 (4): 685-92. PMID 1835632
6. Grundy, S. M., Benjamin, I. J., Burke, G. L., Chait, A., Eckel, R. H., Howard, B. V., ... Sowers, J. R. (1999). Diabetes and cardiovascular disease: A statement for healthcare professionals from the American heart association. *Circulation*, 100(10), 1134-1146 [7] P. Fresneau, M. Cussac, J.-M. Morand, B. Szymonski, D. Tranqui and G. Leclerc, (1998); *J. Med. Chem.*, 41 (24), 4706- 4715
7. Robinson Jr WG., Nagata M, Kinoshita JH. Aldose reductase and retinal capillary basement membrane thickening. *Experimental Eye Research*. Volume 46, Issue 3, March 1988, Pages 343-348

8. Tomlinson, D. R., Willars G. B. and Carrington, A. L. (1992). Aldose reductase inhibitors and diabetic complications. *Pharmacol. Ther.*, 54(2), 151–199.
9. Herbal medicine–its use in treating some symptoms of AIDS; 9th International AIDS Conference.
10. Report: Indigenous Approached to the HIV/AIDS Scourge in Uganda, chapter 5, archived September 28, 2007 at the Wayback Medicine.
11. Traditional Medicine Development for Medical and Dental Primary Health Care delivery system in Africa', African Journal of Traditional Complementary and Alternative Medicines. Volume 2, issue 1, 2005, pages 46-61.
12. Udoamaka F. Ezuruike and Jose M. Prieto (2014). 'The use of plants in the traditional management of diabetes in Nigeria, Pharmacological and toxicological considerations' Journal of Ethnopharmacology, volume 155, issue 2, pg:857–924.
13. Akah, P.A., and Okafor, C.L., (1992). 'Blood sugar lowering effect of *Vernonia amygdalina* Del, in an experimental rabbit model'. Phytotherapy Research volume 6, pg 171–173.
14. Ong, K.W., Hsu, A., Song, L., Huang, D., Tan, B.K.H., (2011). 'Polyphenols-rich *Vernonia amygdalina* shows anti-diabetic effects in streptozotocin-induced diabetic rats'. Journal of Ethnopharmacology volume 133, pg 598–607.
15. Ejike, C.E.C.C., Awazie, S.O., Nwangozi, P.A., and Godwin, C.D., (2013). 'Synergistic post-prandial blood glucose modulatory properties of *Vernonia amygdalina* (Del.), *Gongronema latifolium* (Benth.) and *Occimum gratissimum* (Linn.) aqueous decoctions. Journal of Ethnopharmacology, volume 149, pg 111–116.
16. Hayman, S. and Kinoshita, J. H. (1965); 'Isolation and properties of lens aldose reductase', *J. Biol. Chem.*, 240, 877–82.
17. Ward, W. H. J., Sennitt, C. M., Ross, H., Dingle, A., Tinms, D., Mirrlees, D. J., and Tuffin, D. P., (1990); 'Ponalrestat: a potent and specific inhibitor of aldose reductase', *Biochem. Pharmacol.*, 39: 337.
18. H. Y. Ma, H. Y. Gao, L. Sun, J. Huang, X. M. Xu and L. J. Wu (2011); 'constituents with alpha-glucosidase and advanced glycation end-product formation inhibitory activities from *Salvia miltiorrhiza*' *Bge, J. Nat. Med.* 65, 37–42.
19. M. Pérez, F. J. Muñoz, E. Muñoz, M. Fernández, J. V. Sinisterra and M. J. Hernáiz (2008); 'Synthesis of novel glycoconjugates and evaluation as inhibitors against β -glucosidase from almond', *J. Mol. Catal. B: Enzym.* 52–53, 153-157.
20. Lee LMY, Salvatore AK, Flanagan PR, Forstner GG. Isolation of a Detergent-Solubilized Maltase/Glucoamylase from Rat Intestine and its Comparison with a Maltase/Glucoamylase solubilized by Papain. *Biochem J* 1980;187:437-446.
21. Flanagan PR, Forstner GG. Purification of Rat Intestinal Maltase/Glucoamylase and its Anomalous Dissociation either by Heat or by Low pH. *Biochem J* 1978;173:553-563.
22. Tanaka A, Ohya M, Yamamoto T, Nakagawa C, Tsuji T, Senoo K, Obata H, Steady-State Inhibitory Kinetics Studies on the Ligand Binding Modes of *Aspergillus niger* Glucoamylase. *Biosci Biotechnol Biochem* 1999;63:1548-1552.
23. D. Rakowitz, R. Maccari, R. Ottana and M. G. (2006); 'Vigorita', *Bioorg. Med. Chem.*, 14, 567–574; (b) S. Ali, A. Saeed, N. Abbas, M. Shahid, M. Bolte and J, (2012); Iqbal, *Med. Chem. Commun.*, 2012, 3, 1428–1434.
24. Rammohan Subramanian, M. Zaini Asmawi and Amirin Sadikun (2008). 'In vitro α -glucosidase and α -amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide' *Acta Biochimica Polonica*, volume 55, No: 2, pg:391-398
25. Sindhu. S. Nair, Vaibhavi Kavrekar and Anshu Mishra (2013). 'In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts' *European Journal of Experimental Biology*, volume 3(1):128-132.
26. R.M. Balaji, Chitra Jeyaram, K. Meenakshi, Sundaram and M.S. Ramasamy, (2015). 'Studies on Antidiabetic Activity of Indian Medicinal Plants Using α -Amylase and α -Glucosidase Inhibitory Activity - A Pathway to Antidiabetic Drugs' *World Journal of Medical Sciences* 12 (3): 207-212.
27. N. Verma, B. C. Behera and B. O. Sharma (2012); 'Glucosidase inhibitory and radical scavenging properties of lichen metabolites salazinic acid, sekikaic acid and usnic acid'. *Hacett. J. Biol. Chem.* 40, 7–21.
28. T. Sander, OSIRIS property explorer. Allschwil: Actelion Pharmaceuticals Ltd 2001.