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ORIGINAL ARTICLE

Antioxidant and Inhibitory Effects of Saponin Extracts from *Dianthus basuticus* Burtt Davy on Key Enzymes Implicated in Type 2 Diabetes *In vitro*

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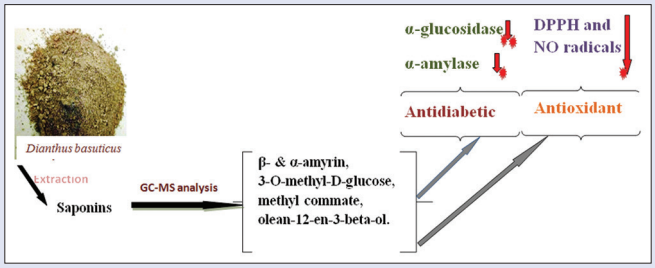
ABSTRACT

Context: *Dianthus basuticus* is a plant of South African origin with various acclaimed pharmaceutical potentials. **Aims:** This study explored the antioxidant and antidiabetic activities of saponin extract from *D. basuticus in vitro*. **Materials and Methods:** Antioxidant activity of saponin was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (*NO)-free radical scavenging activity while antidiabetic potentials were measured by the α-amylase and α-glucosidase inhibitory activities of the saponin extract. **Results:** The results showed that the saponin extract, compared with quercetin, displayed better DPPH (IC_{50} = 6.95 mg/ml) and NO (IC_{50} = 3.31 mg/ml) radical scavenging capabilities. Similarly, the saponin extracts elicited stronger α-glucosidase (IC_{50} = 3.80 mg/ml) and moderate α-amylase (IC_{50} = 4.18 mg/ml) inhibitory activities as compared to acarbose. Saponin exhibited a competitive mode of inhibition on α-amylase with same maximum velocity (Vmax) of 0.0093 mM/min for saponin compared with control 0.0095 mM/min and different the Michaelis constant (Km) values of 2.6 × 10^{-6} mM and 2.1 × 10^{-5} mM, respectively, while for α-glucosidase, the inhibition was uncompetitive, Vmax of 0.027 mM/min compared with control 0.039 mM/min and Km values of 1.02 × 10^{-6} mM and 1.38 × 10^{-6} mM, respectively. The gas chromatography-mass spectrometric analysis revealed the presence of bioactive like β- and α-amyrin, 3-O-methyl-D-glucose, methyl commate, and olean-12-en-3-beta-ol. **Conclusion:** Overall, the data suggested that the saponin extract from *D. basuticus* has potentials as natural antioxidants and antidiabetics.

Key words: Antidiabetics, antioxidants, *Dianthus basuticus*, saponin

SUMMARY

- Saponin extract from *Dianthus basuticus* displayed promising antidiabetic and antioxidant activity
- Saponin competitively and uncompetitively inhibited α-amylase and α-glucosidase, respectively
- The stronger inhibition of α-glucosidase and moderate inhibition of α-amylase by saponin extract from *D. basuticus* is promising good antidiabetes compared with existing drugs with associated side effects.



Abbreviations used: DPPH: 2,2-diphenyl-1-picrylhydrazyl, Km: The Michaelis constant, Vmax: Maximum velocity, ROS: Reactive oxygen species, NIDDM: Non-insulin-dependent diabetes mellitus, UFS: University of the Free State, GC-MS: Gas chromatography-mass spectrometric, MS: Mass spectrometry, NIST: National Institute of Standards and Technology, DNS: 3,5-dinitrosalicylic acid, NO: Nitric oxide, RNS: Reactive nitrogen species, PNPG: p-Nitrophenyl-α-D-glucopyranoside.

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INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder, characterize by high blood sugar due to total or partial insulin insufficiency,[1] with interruptions in carbohydrate, fat, and protein metabolism.[2] Diabetes is one of the largest global health crises of the 21st century. Some 415 million people worldwide or 8.8% of adults aged 20–79 years are projected to have diabetes. Estimated 14.2 (9.5–29.4) million adults aged 20–79 years experience diabetes in the Africa region, accounting for a regional incidence of 2.1%–6.7%. Some of Africa's most populous nations have the uppermost occurrences of diabetes, including South Africa (2.3 million), Democratic Republic of the Congo (1.8 million), Nigeria (1.6 million), and Ethiopia (1.3 million), and by 2040, some 642 million people or one adult in ten will have diabetes.[3]

Diabetes is linked with high oxidative stress and decreased antioxidant conditions.[4] During diabetic states, reactive oxygen species (ROS) are generated largely during the glycation reaction,[5,6] which arises in different tissues[7] and may play important part in the development of problems in diabetes.[8] Latest findings showed that high blood sugar may possibly provoke nonenzymatic glycosylation of diverse macromolecules, production of free radicals, and change of endogenous antioxidants that may cause the manifestation of chronic complications in diabetes.[9,10]

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One curative approach for treating diabetes is to reduce the postmeal rise in blood glucose in diabetic patients. This is achieved by impeding the uptake of sugar by inhibiting the gastrointestinal carbohydrate-degrading enzymes, α-glucosidase and α-amylase. Inhibitors of these enzymes interrupt carbohydrate breakdown and elongate overall carbohydrate pace of digestion, thereby slowing down the rate of glucose absorption and accordingly blunting the postprandial plasma glucose increase.[11] α-glucosidase has been identified as a therapeutic object for the maintenance of high blood sugar after meal, which is the initial metabolic irregularity that happens in noninsulin-dependent diabetes mellitus (NIDDM).[12] The main supply of blood glucose is dietary carbohydrates such as starch, which are broken down by α-glucosidases and pancreatic α-amylase, to facilitate absorption by the small intestine. Therefore, a successful treatment option for NIDDM is to restrain the activity of α-glucosidases and pancreatic α–amylase.[13] Incidentally, inhibitors can slowdown the absorption of dietary carbohydrates, stem postprandial hyperglycemia, and could be useful for the management of diabetes and/or obesity in patients.[14] Some α-glucosidase inhibitors such as acarbose, miglitol, and voglibose are identified to diminish postprandial rise in blood glucose mainly by impeding the activity of carbohydrate-digesting enzymes and holding up glucose absorption.[15] These inhibitors have been identified to cause several side effects, such as abdominal distention, flatulence, meteorism, and possibly diarrhea.[16] A large number of traditional medicinal plants and plant-derived components have been reported to possess α-glucosidase and α-amylase inhibitory activity.[17] Thus, natural products of great structural diversity are still a good source for searching for such inhibitors, thereby motivating to explore biologically active compounds from the highly diverse plants.[18]

Saponins have both water-soluble and lipid-soluble parts. They compose of a lipid-soluble nucleus, with either a steroid or a triterpenoid aglycone structure, with one or more side chains of water-soluble carbohydrates.[19] Due to the presence of a lipid-soluble aglycone and water-soluble sugar chain in their structure (amphiphilic nature), saponins are surface-active compounds with detergent, wetting, emulsifying, and foaming properties.[20] Information on pharmacological activities exhibited by some isolated saponins comprises hypocholesterolemic, anticarcinogenic, antioxidant, hypoglycemic, and antiprotozoans.[21] Saponins have recognized with wide relevancies in beverages and confectionaries and in cosmetics.[22-24]

Dianthus basuticus are evergreen soft-wooded perennials with brilliant pink flowers and intensely fringed. It is found on Drakensberg Mountains in South Africa and also found in Lesotho, Cape, KwaZulu-Natal, Free State, and Northern provinces (rocks and grassland).[25] It is commonly called Lesotho carnation and the decoction used for cleansing of blood flatulence and fertility in bulls.[26] The roots is masticated as magic to preserve a loved one's affection.[27] Previously reported scientific investigations were based on antimicrobial and cytotoxic potentials of *D. basuticus*,[27] safety evaluation of in animal model,[28] and the *in vitro* antioxidant and antidiabetic potentials of whole-plant extracts.[29] The plant has also been reported to be rich in phytochemical constituents such as saponins, alkaloids, tannins, and cardiac glycosides.[27] The current investigation was carried out *in vitro* α-glucosidase and α-amylase inhibitory potentials of saponin extract from *D. basuticus* and its free radical scavenging activities.

MATERIALS AND METHODS

Chemicals and reagents

Acetone, chloroform, ethanol, petroleum ether, and methanol are products of LASEC, South Africa. Rat intestinal α-glucosidase, porcine pancreatic α-amylase, p-nitrophenyl alpha-D-glucopyranoside, 1,1-diphenyl-2-picrylhydrazyl, quercetin, and acarbose were produced by Sigma-Aldrich Co., St. Louis, USA, while starch was obtained from J. T. Baker Inc., Phillipsburg, USA. Additional reagents were of analytical grades and prepared in glass-distilled water.

Plant materials

The plant material (whole plant, i.e., aerial parts and the roots) was gathered in January 2013 from a field around Qwaqwa inside the Golden Gate Mountains (280 28//111/S and 280 48//314/E; altitude 11950 m). The species' great quantity was taken into consideration, and collections were done in such a way that the survival of the species was not in danger. Authentication was carried out at the Bews Herbarium of the University of Kwazulu-Natal, Pietermaritzburg Campus, by Dr. C. J. Potgieter. A reference sample with herbarium voucher number (LamMed/01/2013/Qhb) was previously dropped at the UFS-Qwaqwa campus herbarium.

Saponin extraction procedure

The extraction process was carried out with slight modification from previous method reported by Lakshmi *et al.*[30] It involved soaking 30 g of the dried plant sample in 200 ml of 95% ethanol overnight. Petroleum ether, ethyl acetate, chloroform, methanol, and acetone were used for the extraction. While petroleum ether was used for delipidization, chloroform was used for deproteinization of dried mixture. Methanol was used to mellow the developing mixture during the extraction of crude saponin, after which the concentrated solution was added drop wisely into acetone solution leading to precipitation. Thereafter, precipitate was dried in oven (37°C) leading to formation of whitish-brown crystals. Frothing test was carried out to confirm the saponin. Small quantity of the extract was dissolved in distilled water, and the test tube was agitated briskly for 30 s. It was left in upright position and watched for 30 min. Substantial constant froth was observed on the surface of the liquid showing the presence of saponin. The saponin extract was dissolved in distilled water to give stock solutions of 1.0 mg/ml and various concentrations (0.063, 0.125, 0.25, 0.50, and 1.0 mg/ml) of the saponin extract done using a serial dilution technique with distilled water. All extracts were thereafter stored at 4°C before analysis.

Gas chromatography-mass spectrometric analysis of the saponin extract

Saponin from *D. basuticus* subjected to gas chromatography-mass spectrometric (GC-MS) examination by an Agilent Technologies 6890 Series gas chromatograph together with (an Agilent) 5973 Mass Selective detector and determined by Agilent Chemstation software. An eHP-5MS capillary column was used (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250°C. The initial oven temperature was at 60°C, which was programmed to move up to 280°C at the rate of 10°C/min with a hold time of 4 min at each increment. Injections of 2 μL were done in the splitless mode with a split ratio of 20:1. The mass spectrometer was derived in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadruple temperature 150°C, solvent delay 4 min, and scan range 50–700 amu. The compounds were recognized by direct comparison of the retention times and mass spectral data and fragmentation prototype with those in the Wiley Library and National Institute of Standards and Technology (NIST) library.

Antidiabetic assay

α-amylase inhibition assay

The assay was done as illustrated by Shu *et al.*[31] with some modifications. Briefly, 50 µl of saponin sample in 0.02 M phosphate buffer (pH 6.9, containing 0.006 M NaCl) was mixed with 25 µl of enzyme solution (0.5 mg/ml) and incubated at 25 ± 1°C for 10 min. Thereafter, 50 µl of starch solution (0.5% (w/v), 0.02 M phosphate buffer, pH 6.9, containing 0.006 M NaCl) was added and incubated for 10 min at 25 ± 1°C. Then, 100 µl 3,5-dinitrosalicylic acid (DNS) color reagent was added to stop the reaction (100 mg DNS dissolved in 3 ml distilled water, to this 3 g $KNaC_4H_4O_6.4H_2O$, and 2 ml of 2 M NaOH were added; the solution was diluted to 10 ml with distilled water) and immediately kept in water at 100°C in a water bath for 10 min. The absorbance of the resultant solution was taken at 540 nm. The uninhibited enzyme was taken as control. Suitable blank was used for all the samples. Acarbose was used as the standard inhibitor of the enzyme.

Mode of α-amylase inhibition

The mode of inhibition of α-amylase by the saponin was determined following the modified technique used Ali *et al.*[32] In brief, 250 µl of the (1.00 mg/ml) saponin extract was initially incubated with 250 µL of α-amylase solution for 10 min at 25°C test tubes. In a new set of tubes, α-amylase was preincubated with 250 µl of phosphate buffer (pH 6.9). Two hundred and fifty microliter of starch solution at ascending concentrations (0.025–0.40 mg/ml) was added to start the reaction. The mixture was then incubated for 10 min at 25°C and then boiled for 5 min following addition of 500 µl of DNS to end the reaction. The amount of reducing sugars freed was established spectrophotometrically with maltose standard curve and changed to reaction velocities. Lineweaver–Burk graph (1/v vs. 1/[S]) where [S] indicates substrate concentration and V represents reaction velocity was plotted to establish the mode of inhibition.

α-glucosidase inhibitory assay

The slightly modified method of McCue and Shetty[33] was followed. In brief, in a 96-well microplate, 50 µl α-glucosidase (1.0 U/ml) in phosphate buffer (0.1 M, pH 6.8) for 10 min at 37°C was incubated with 50 µl of saponin of various concentrations (0.625–1.0 mg/ml). The reaction was prompted by addition of 50 µl of 5 mM, *p*-Nitrophenyl-α-D -glucopyranoside in a 0.1 M phosphate buffer at pH 6.8. P-nitrophenol's release kinetics were read with a microplate spectrophotometric reader Multiskan MS™ (Labsystems, Minneapolis, USA) for 5 min at intervals of 30 s and absorbance was measured at 405 nm. Acarbose served as the reference standard. The minimum inhibitory concentration of the extract IC_{50} was then estimated from the concentration-dependent graph.

Mode of α-glucosidase inhibition

The mode of inhibition of α-glucosidase by saponin was assessed with the procedure explained by Ali *et al.*[32] In brief, 50 µL of the (0.063–1.00 mg/ml) saponin extract was initially incubated with 100 µL of α-glucosidase for 10 min at 25 ^{0}C in a set of tubes. α-glucosidase was preincubated with 50 µL of phosphate buffer (pH 6.9) in another set of tubes. Fifty microliter of p-Nitrophenyl-α-D-glucopyranoside at ascending concentrations (0.025–0.40 mg/ml) was added to initiate the process. The mixture was thereafter incubated for 10 min at 25°C, and 500 µL Na_2CO_3 was added to end the reaction. The quantity of reducing sugars released was estimated spectrophotometrically by a para-nitrophenol standard curve and changed to reaction velocities. A Lineweaver–Burk graph (1/v vs. 1/[S]) where [S] represents substrate concentration and v stands for reaction velocity was plotted to establish the type of inhibition.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The antioxidant activity of the saponin was determined by measuring its capacity of bleaching the purple-colored ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described by Turkoglu *et al.*[34] In brief, 100 µl of different concentrations (0.625–1.0 µg/ml) of the saponin extract in methanol mixed with 100 µl of 0.2 mmol/L DPPH in methanol. After 30 min incubation period at ambient temperature, the absorbance was measured at 516 nm. The rate of inhibition rate (I %) on the DPPH radical was evaluated with the expression:

Percentage Inhibition (I %) = $[(A_{control} - A_{extract})/A_{control}] \times 100$,

Where $A_{control}$ is the absorbance of the control, $A_{extract}$ is the absorbance of the extract.

Nitric oxide scavenging assay

Nitric oxide (NO) radical halting activity of the saponin was examined by the method of Garrat.[35] In briefly, 100 µl of 10 M sodium nitroprusside in 0.5 mL phosphate-buffered saline (pH 7.4) was mixed with 50 µl of varying concentrations of saponin extract and incubated at 25°C for 2 h. Afterward 50 µl was withdrawn and poured into100 µl sulfanilic acid reagent (33% in 20% glacial acetic acid) from the incubated mixtures and incubated further at room temperature for 5 min. After this, 1 ml N-(1-Naphthyl)ethylenediamine dihydrochloride (0.1% w/v) was added into the mixtures and the resultant solution incubated at room temperature for 30 min. The absorbance was read at 540 nm and the minimum inhibitory concentration (IC_{50}) was then extrapolated from the calibration curve after the evaluation of percentage NO radical scavenging capacity of CSE using the expression:

Percentage scavenging (S %) = $[(A_{control} - A_{extract})/A_{control}] \times 100$,

Where $A_{control}$ is the absorbance of the control, $A_{extract}$ is the absorbance of the extract.

RESULTS

The percentage yield of the saponin was 8.6%. The DPPH and NO radical scavenging activities of the saponin extract are presented in Figures 1 and 2. In both cases, the saponin produced higher DPPH and NO radical mopping up potential than quercetin. The IC_{50} of DPPH (6.95 mg/ml) and NO (3.31 mg/ml) when compared with that of quercetin 14.69 mg/ml and 3.67 mg/ml, respectively, are clear indicators [Table 1].

Figures 3 and 4 represent the results of inhibition of α-amylase and α-glucosidase by the saponin extract of *D. basuticus*. Compared with

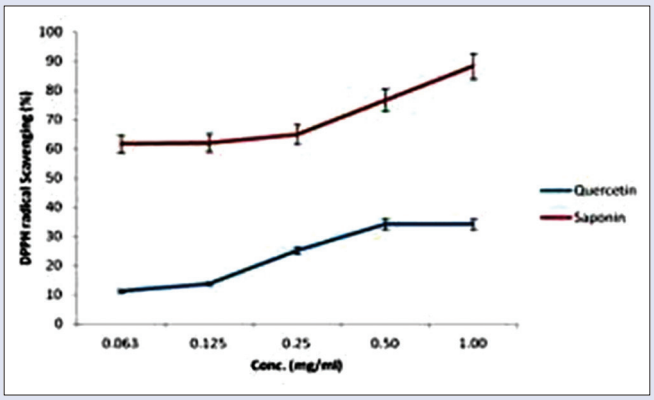

Figure 1: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of saponin extract from *Dianthus basuticus*

the acarbose, saponin displayed superior inhibition of α-glucosidase and mild inhibition of α-amylase. The inhibitory concentration (IC_{50}) of saponin extract [Table 2] obtained was 3.80 mg/ml (α-glucosidase) and 4.18 mg/ml (α-amylase) compared with acarbose 6.27 mg/ml and 2.34 mg/ml, respectively.

The mode of inhibition of the enzymes is as indicated in Figures 5 and 6. Saponin displayed a competitive mode of inhibition on α-amylase with same maximum velocity (Vmax) of 0.0093 mM/min for saponin compared with control 0.0095 mM/min and different the Michaelis constant (Km) values of 2.6×10^{-6} mM and 2.1×10^{-5} mM, respectively, while for α-glucosidase, the inhibition was uncompetitive, both Vmax and Km were different, saponin Vmax was 0.027 mM/min compared with control 0.039 mM/min and Km values of 1.02×10^{-6} mM, 1.38×10^{-6} mM respectively.

GC-MS analysis of the saponin extract from *D. basuticus* [Figure 7, Table 3 and 4] revealed the presence of some potential antidiabetic and antioxidant constituents when compared with standard mass spectra in the Wiley Library and NIST library. Compounds such as mome inositol, 3-O-methyl-d-glucose, β- and α-amyrin, urs-12-en-3-ol, and olean-12-en-3-beta-ol among others were identifiable constituents.

Table 1: Inhibitory concentration 50% of saponin from *Dianthus basuticus* on 2,2-diphenyl-1-picrylhydrazyl and nitric oxide radicals

	DPPH		NO	
	Quercetin	Saponin	Quercetin	Saponin
IC_{50} (mg/mL)	14.69	6.95	3.67	3.31

IC_{50}: Inhibitory concentration 50%; DPPH: 2,2-diphenyl-1-picrylhydrazyl; NO: Nitric oxide

Table 2: Inhibitory concentration 50% of saponin from *Dianthus basuticus* on α-amylase and α-glucosidase

	α-amylase		α-glucosidase	
	Acarbose	Saponin	Acarbose	Saponin
IC_{50} (mg/mL)	2.34	4.18	6.27	3.80

IC_{50}: Inhibitory concentration 50%

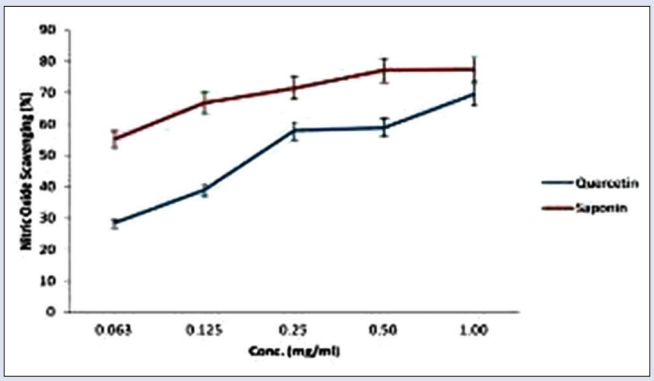

Figure 2: Nitric oxide scavenging activity of saponin extract from *Dianthus basuticus*

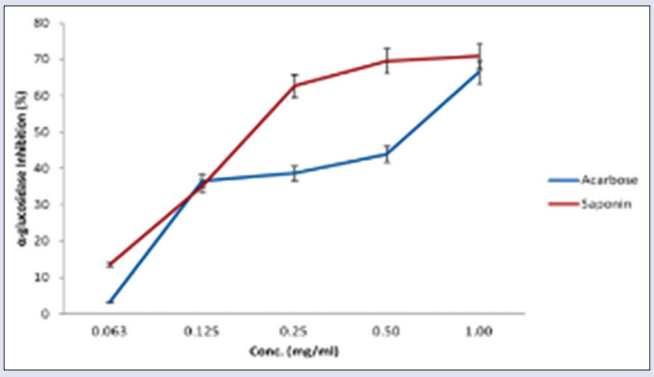

Figure 4: α-glucosidase inhibitory activity of saponin extract from *Dianthus basuticus*

DISCUSSION

Several reports have revealed that diabetes mellitus is linked to rise in the generation of ROS and reduced scavenging ability. Consequently, the normal cellular equilibrium in the production and mopping up capacity is compromised. This results to oxidative destruction of cellular constituents such as proteins, lipids, and nucleic acids due to increased oxidative stress.[36] Increased oxidative stress in diabetes is due to various factors. Prominent of these factors is auto-oxidation of glucose resulting to formation of free radicals. In addition, it is lowered antioxidant defense and imbalances in cellular oxidation/reduction reactions.[37] Furthermore, antioxidant mechanisms are reduced in diabetic patients who promote oxidative stress.[38,39]

Traditional herbal medicines are naturally occurring plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices.[40] Plant phytochemical possesses antioxidant activities which are demonstrated by halting the formation of free radicals or by counteracting/scavenging

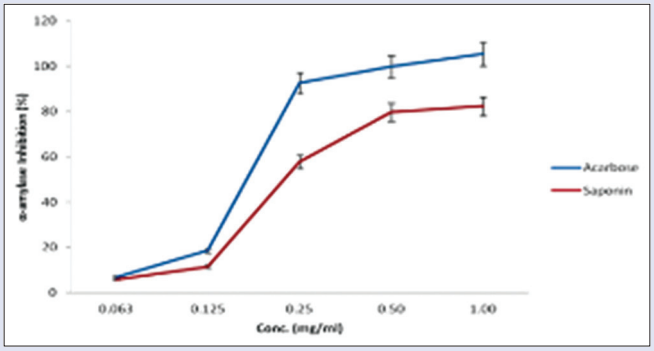

Figure 3: α-amylase inhibitory activity of saponin extract from *Dianthus basuticus*

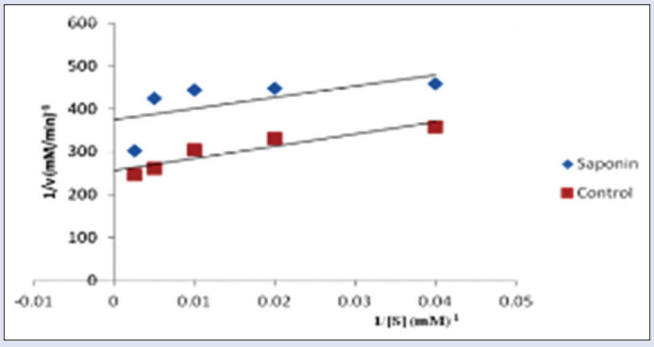

Figure 5: Modes of inhibition of α-glucosidase by saponin extract from *Dianthus basuticus*

free radicals generated in the body.[41] DPPH radical scavenging technique is very significant and has been extensively used for assessing antioxidant potentials in many investigations.[42] The principle of DPPH procedure depends on the lowering of DPPH in the presence of a proton-releasing antioxidant. A lot of naturally occurring antioxidants have been shown to exhibit major roles in stemming both free radicals and oxidative chain reactions within tissues and membranes.[43] The results from this study demonstrated the ability of saponin from *D. basuticus* to scavenge DPPH radicals. The IC_{50} of the saponin (6.95 mg/ml) compared with quercetin (standard) (14.69 mg/ml) clearly suggested a superior scavenging power of saponin from *D. basuticus*. The result of this study corroborates the earlier report of Kazeem and Ashafa[29] on the DPPH scavenging activity of aqueous extract of *D. basuticus* and is in conformity with the report of Akinpelu *et al.*[44] on the antioxidant activity of saponin fraction from E*rythrophleum suaveolens*. This implies that saponin extract from *D. basuticus* has the proton-donating capacity and could serve as inhibitor of free radical and probably as a primary antioxidant.

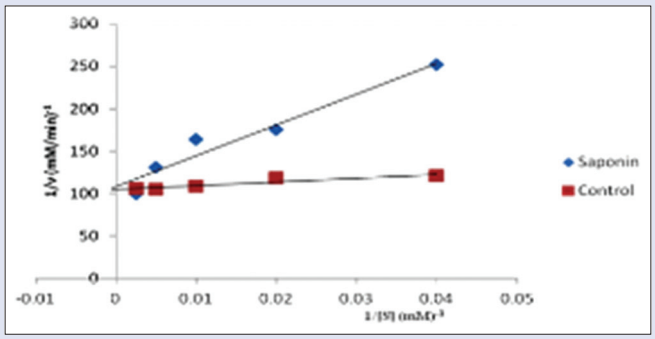

Figure 6: Modes of inhibition of α-amylase by saponin extract from *Dianthus basuticus*

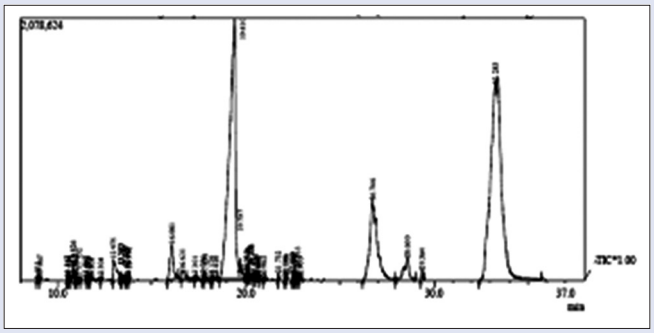

Figure 7: Gas chromatography-mass spectrometric chromatogram of the saponin extract of *Dianthus basuticus*

Table 3: Some compounds identified in the gas chromatography-mass spectrometric analysis of saponin extract of *Dianthus Basuticus*

Retention time	Peak area (%)	Active compounds	Molecular formula
19.410	36.65	Mome inositol	$C_7H_{14}O_6$
		3-O-methyl-d-glucose	$C_7H_{14}O_6$
		α-d-mannopyranoside	$C_7H_{14}O_6$
		Methyl-hexafuranoside	$C_7H_{14}O_6$
26.746	12.31	Norolean-12-ene	$C_{29}H_{48}$
		α-amyrin, α-amyrenol, α-amyrine	$C_{30}H_{50}O$
		Urs-12-en-3-ol	$C_{30}H_{50}O$
		Viminalol	$C_{30}H_{50}O$
		Octamethayl-1, 4-derivatives	$C_{30}H_{48}O$
		Methyl commate	$C_{32}H_{52}O_4$
33.283	45.14	β-amyrin, β-amyrenol, β-amyrine	$C_{30}H_{50}O$
		Olean-12-en-3-beta-ol, acetate	$C_{32}H_{52}O_2$
		β-amyrenyl acetate, β-amyrin acetate	$C_{32}H_{52}O_2$
		3-keto-urs-12-ene	$C_{30}H_{48}O$

Table 4: Activities of some phytocomponents identified in the saponin extract of *Dianthus Basuticus*

Compound	Type	Bioactivities
Mome inositol	Polysaccharide	Antiproliferative,[51] anticirrhotic, lipotropic, antialopecic, antineuropathic, cholesterolytic, and a sweetener[51,52]
β- and α-amyrin	Triterpenes	Analgesic, anti-inflammatory,[53] Antibacterial, antifungal, anti-inflammatory and antiulcer[54]
Methyl commate	Triterpenes glycoside	Antibacterial, antimicrobial, insecticides, nematicides, and are highly effective in wound healing activities[55]
3-O-methyl-D-glucose	Polysaccharide derivative	It is a nontoxic nonmetabolizable derivative of glucose, is effective in reducing the toxicity of SZ. It has been found to possess antitumor, oncogenic, and diabetogenic properties.[56] It is quickly absorbed into cells,[57,58] and it concentrates due to its not metabolizable. It has been applied as a cryoprotectant for the cryopreservation of liver cells[56] and for enhancing desiccation tolerance of keratinocytes[60]
Urs-12-en-3-ol, acetate, and 3-keto-urs-12-ene	Triterpenes	Antitumor, antiviral, anti-inflammatory, hepatoprotective, gastroprotective, antimicrobial, antidiabetic, and hemolytic properties[61]
Olean-12-en-3-beta-ol	Triterpenes	Antioxidant, antiproliferative, attenuation of myocardial apoptosis, beneficial effects on oxidative stress, and inflammation, reduced blood cholesterol levels[62]

SZ: Streptozotocin

NO can permeate membranes freely or work on several cellular targets. It acts as moderator of various physiological activities such as vasorelaxation, macrophage activation, gene expression, and apoptosis and typically taken as a vasculoprotective molecule.[44] However, one of its several properties is protein nitrosylation at the thiol groups as well as RNS generation like peroxynitrite ($ONOO^-$) as *NO easily reacts with $\bullet O_2$-. Thus, the number of $^\bullet O_2$- determines if $^\bullet$NO acts as a defensive or damaging molecule.[45,46] NO supplies practical information on the reactivity of the compound production from sodium nitroprusside and measured by the Griess reaction. Scavengers of NO contend with oxygen ensuing lowered formation of NO.[47] NO radicals were inhibited by saponin extract from the root of *D. basuticus*. Saponin extract displayed a fairly better *NO scavenging strength, IC_{50} (3.31 mg/ml) compared with the standard (quercetin), IC_{50} (3.67 mg/ml). This result also concurred with the studies of Alli-Smith and Adanlawo[47] on the saponin extract from the root of *Garcinia kola*.

In patients with diabetes, high blood sugar is prominent following a meal due to the absorption of glucose from the digestive tract.[48] Complex carbohydrates are broken down by intestinal α-amylase to oligosaccharide which is thereafter hydrolyzed to glucose by intestinal α-glucosidase previous to being absorbed into the intestinal epithelium and diffusing into blood circulation.[49] Thus, inhibition of glucose formation and/or advancing glucose removal in the tissues may be helpful for those patients to control the hyperglycemia in the postprandial state.[48] An effective way to prevent postmeal upsurge in the blood glucose is the inhibition of α-glucosidase and α-amylase activity. Our results displayed that saponin extract forms *D. basuticus* having inhibitory potentials on these enzymes. *D. basuticus* saponin extract strongly inhibited α-glucosidase activity [Figure 4] and mild inhibition of α-amylase activity [Figure 3]. The inhibitory activity elicited by saponin, IC_{50}, 3.80 mg/ml on α-glucosidase was stronger than that of the standard, acarbose, IC_{50}, 6.27 mg/ml., while in the case of α-amylase, acarbose, IC_{50}, 2.34 mg/ml displayed a better inhibition than saponin extract, IC_{50}, 4.18 mg/ml. These results are further consolidation of folkloric antidiabetic use of *D. basuticus* and consistent with the earlier reported inhibitory potentials of the various fractions of the plants by Kazeem and Ashafa[29] on the α-glucosidase and α-amylase activities *in vitro*. Higher α-glucosidase inhibitory activities of saponin extract from *D. basuticus* over that of its corresponding α-amylase have been reported to be of great pharmaceutical significance in addressing some of the side effects linked to acarbose and voglibose applied for the treatment of type 2 diabetes that are associated with excess inhibition of α-amylase.[16]

The competitive mode of inhibition displayed by the saponin extract on α-amylase was an indication that the saponin competed with the substrate at the active site of the enzyme. The implication of this is that by increasing the concentration of the substrate, the inhibition can be reversed.[50] Conversely, the uncompetitive inhibition of the saponin on α-glucosidase indicates that saponin binds only to enzyme–substrate complex at locations aside the catalytic site. Thus, there is modification of the enzyme structure, rendering inhibitor-binding position accessible, and in this case, inhibition cannot be reversed by substrate.[50] The mode of inhibition of saponin extract on both enzymes further confirmed the mild inhibition noted for α-amylase and strong inhibition obtained for α-glucosidase. This further consolidates saponin extract from *D. basuticus* as having great pharmaceutical significance in tackling some of the problems related to known standard drugs.

CONCLUSION

As efforts to source for alternative antidiabetic are still ongoing, the result obtained from the current study is an indicator of possible success in the near future. The outcome of this study displayed promising potentials of saponin extract from *D. basuticus* as a possible antidiabetic drug candidate source. More efforts are ongoing to purify, characterize, and evaluate the toxicity profile of the saponin from this plant.

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Conflict of interest

There are no conflicts of interest.

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