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%

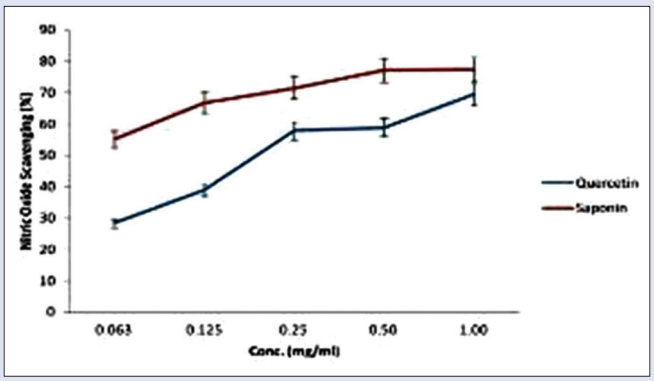

Figure 2: Nitric oxide scavenging activity of saponin extract from *Dianthus basuticus*

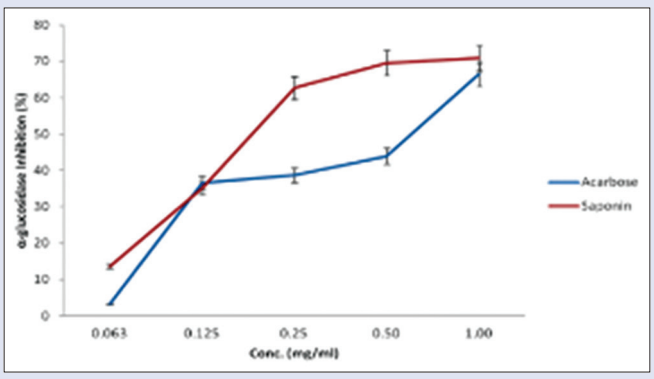

Figure 4: α-glucosidase inhibitory activity of saponin extract from *Dianthus basuticus*

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

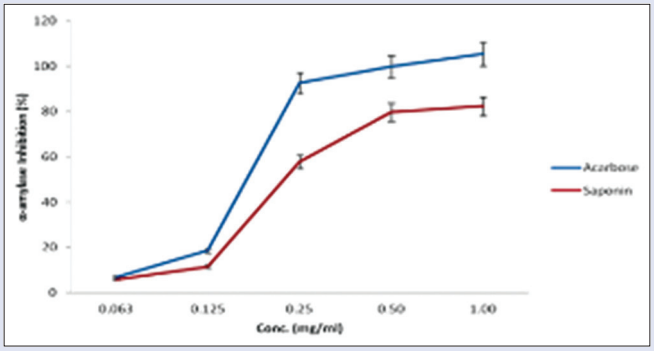

Figure 3: α-amylase inhibitory activity of saponin extract from *Dianthus basuticus*

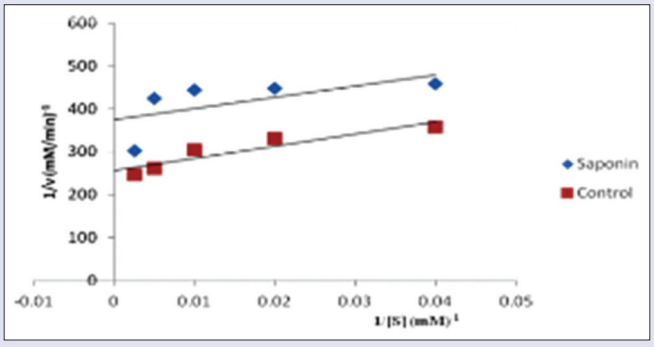

Figure 5: Modes of inhibition of α-glucosidase by saponin extract from *Dianthus basuticus*

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.

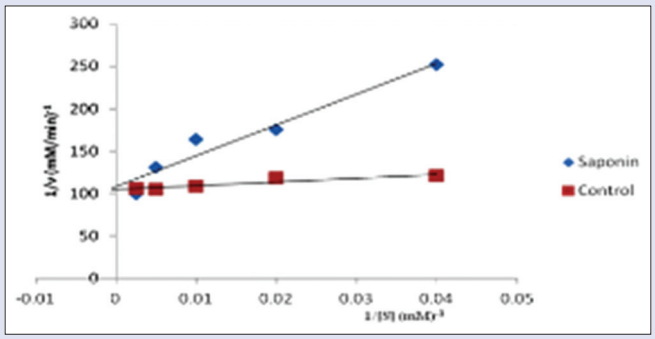

Figure 6: Modes of inhibition of α-amylase by saponin extract from *Dianthus basuticus*

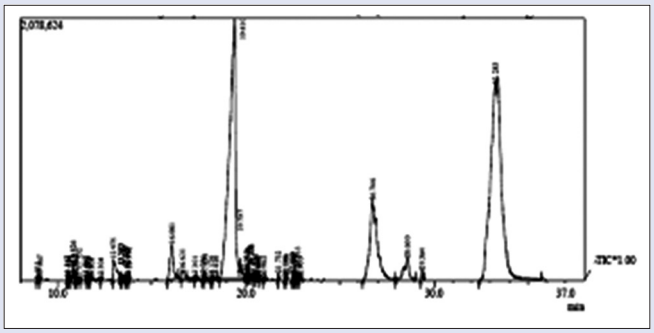

Figure 7: Gas chromatography-mass spectrometric chromatogram of the saponin extract of *Dianthus basuticus*

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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