SCREENING OF *IN VITRO* ANTIDIABETIC EFFECT OF CRUDE EXTRACTS AND ISOLATION OF SOME TERPENOIDS FROM TUBERS OF *DIOSCOREABULBIFERA*L. (HPWUT-SA-U)

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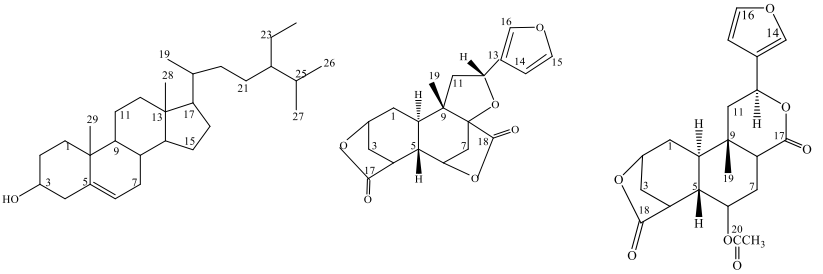
Abstract

*Diabetes mellitus*is a metabolic disorder characterized by chronic hyperglycemia. The management of the blood glucose level is a critical strategy in the control of diabetes complications. Low-cost herbal treatment is recommended due to their lesser side effect for treatment of diabetes. In this paper, tubers of *D. bulbifera* having significant traditional therapeutic potential were tested for their efficiency to inhibit α-amylase and α-glucosidase. *In vitro* α-amylase inhibitory activity of pet-ether, ethyl acetate, ethanol extracts as well as antidiabetic drugs, acarbose and metformin, were determined by starch iodine method and the 50 % inhibitory activity was found to be 172.50 μg/mL, 62.50 μg/mL, 65.00 μg/mL, 60.50 μg/mL and 42.50 μg/mL respectively. *In vitro*α-glucosidase inhibitory activity was measured by glucose oxidase method and the IC_{50} values of acarbose, metformin, pet-ether, ethyl acetate and ethanol were found to be 35.75, 67.75, 137.50, 80.00 and 115.25 μg/mL respectively. From ethyl acetate extract which revealed significant inhibitory activity on both enzymes, compounds A, B and C were isolated by column and thin layer chromatography and identified by modern spectroscopic methods. From these assessments, the isolated compounds were β-sitosterol (A) (0.0412 % yield, m.p 139 °C), diosbulbin B (B) (0.0012 % yield, m.p 217 °C) and 8-epidiosbulbin E-acetate (C) (0.0210 % yield, m.p 225 °C).



β-sitosterol (**A**) diosbulbin**B** (**B**) 8-epidiosbulbin E-acetate(**C**)

Keywords**:** antidiabetic**,** α-amylase, α-glucosidase, β-sitosterol, diosbulbin B,8-epidiosbulbin E-aceta

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Introduction

Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. It is characterized by hyperglycemiain postprandial and fasting state and its severe form is accompanied by ketosis and protein wasting. Evaluated postprandial hyperglycemia is one of the risk factors and it is elevated by the action of α-amylase and α-glucosidase. Inhibitions of these enzymes play a major role in managing postprandial hyperglycemia in diabetic patients. Inhibition of α-glucosidase enzyme activity leads to a reduction in disaccharide hydrolysis and inhibition of carbohydrate hydrolyzing enzyme such as α-amylase lowers blood glucose levels (Qaisar *et al.,* 2014). Many plants and their products have been widely prescribed and used for diabetic treatment all around the world. Thus, these natural products need to be evaluated scientifically in order to verify for their anti-diabetic properties (Bhutkar and Bhise, 2012).

Myanmar is rich in variety of medicinal plants and many people use these plants for their health care. *Diabetes mellitus*is one of the priority diseases in Myanmar. There are numerous indigenous medicinal plants which are reputed to be effective for the treatment of diabetes. Among them, *D. bulbifera* L. was selected from literature review (Gosh *et al.,* 2015). *D. bulbifera* belongs to the family Dioscoreaceae which is distributed in tropical and subtropical regions of Asia. This plant possesses profound therapeutic potential especially in the treatment of sore throat, gastric cancer, carcinoma of rectum and goiter. The various extracts of tubers of this plant have been reported to be antihyperlipidemic, antitumor, antioxidant, analgesic and anti-inflammatory and antihyperglycemic (Gao *et al.,* 2002). The present investigation was undertaken to study the ability of tubers of *D. bulbifera* to inhibit α-amylase and α-glucosidase activity and to isolate some phytoconstituents from it.

Figure 1: The photograph of plant of *D. bulbifera* L.

Figure 2: The photograph of tuber of *D. bulbifera* L.

Materials and Methods

Sample Collection and Preparation

Tubers of *D. bulbifera* used in this study were collected from Mawlamyine University campus. The collected sample was washed with water, air dried and peeled using a stainless knife. Then, the yam tissue was chopped into small cubes which were later air-dried to a constant weight at room temperature for one week. The dried sample was subsequently milled into coarse powder using a grinding mill and then stored in airtight plastic container for the experimental works.

Reagents

α-amylase (Jiangsu BoliBioproducts Co., Ltd), α-glucosidase (Cool Chemical Science and Technology Ltd., Beijing), Glucose oxidase (Hefei-Bomei Biotechnology Co., Ltd), Bicinchoninic acid (BCA), Metformin (DenkPharma GmbH Co., Munchon, Germany), Acarbose (Bayer Pharma AG, Kaiser-Wilhein-Allee, Leverkusen, Germany), Sucrose (BDH), Starch, Iodine, Pet ether, Ethyl acetate, Ethanol, n-hexane

Preparation of crude extracts

The dried powdered sample (300 g) was extracted with pet-ether, ethyl acetate and ethanol respectively by using maceration method. After 72 h

extraction at room temperature, each extract was filtered through Whatman's filter paper No.1 separately. This procedure was repeated for three times. The combined filtrates were evaporated under reduced pressure by means by a rotatory evaporator. Consequently, pet-ether, ethyl acetate and ethanol soluble extracts were obtained.

α-amylase inhibition assay

Alpha-amylase inhibitory activity of plant extracts was determined by starch iodine method (Ganapaty *et al.,* 2013). 10 μL of α-amylase solution (0.025 mg/mL) was mixed with 390 μL of phosphate buffer (40 mM containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts and standard metformin and acarbose. After incubation at 37 °C for 10 min, 100 μL of starch (1 %) was added to the mixture and it was reincubated for 30 min. Then, 0.1 mL of 1 % iodine solution was added to this mixture. After adding 5 mL of distilled water, the absorbance was measured at 565 nm by UV spectrometer. Substrate and α-amylase blank determinations were carried out under the same conditions. The inhibitory activity was calculated by using the formula;

$$\% \text{ inhibition of enzyme activity} = \frac{A - C}{B - C} \times 100$$

where, A = absorbance of the sample

B = absorbance of blank (without α-amylase)

C = absorbance of control (without starch)

The concentration of test samples and standard which inhibited the hydrolysis of starch by 50 % (IC_{50}) were determined by linear regressive excel programme.

α - Glucosidase inhibition assay

α-glucosidaseinhibition activity can be measured *in vitro* by determining the reducing sugar (glucose) arising from hydrolysis of sucrose by α-glucosidase enzyme (Narkhede *et al.,* 2011). For estimating glucose concentration, a glucose oxidase method was used in which glucose-oxidase acts on glucose to produce hydrogen peroxide which directly reduced Cu (II)

complex of 2,-2' bicinchoninic acid (BCA) to Cu (I) complex (Warren, 1990). 75 μL of α-glucosidase solution (0.01 mg/L) was mixed with phosphate buffer (80 mM) containing different concentration of extracts and standard metformin and acarbose. After incubation at 37 °C for 30 min, 500 μL of 40 mM sucrose solution was added and reincubated for 20 min at 37 °C. Then the reaction mixture was kept in boiling water bath for 2 min and cooled at room temperature. After adding 300 μL of glucose-oxidase solution (1 mg/ 1 mL), it was reincubated at 37 °C for 10 min and 2 mL of colouring agent (BCA) was added. Then that solution was incubated for 20 min at 37 °C and the absorbance was measured at 565 nm by UV spectrophotometer. The percentage of inhibition was calculated from the following formula

$$\% \text{ inhibition of enzyme activity} = \frac{A - C}{B - C} \times 100$$

where, A = absorbance of the sample

B = absorbance of blank (without test sample)

C = absorbance of control (without BCA)

IC_{50} value was determined by linear regressive excel programme.

Isolation and identification of phytoconstituents

Among the three extracts, pet-ether, ethyl acetate and ethanol, ethyl acetate extract showed significant inhibition activity on both α-amylase and α-glucosidase enzymes. So part of the EtOAc extract (5 g) was subjected to column chromatography over silica gel using n-hexane:EtOAc as gradient elution to yield five sub-fractions; named I-V. Compounds A (0.0412 % yield) was directly isolated as solid material from fraction I and purified by washing with n-hexane and recrystallizing in chloroform and methanol. Fraction II which showed detectable sorts on TLC was further rechromatographed by using chloroform:methanol (19:1, 9:1 v/v) as eluents and afforded compounds B (0.33 % yield) and C (0.69 % yield).These isolated compounds were characterized by their physicochemical properties; melting point, R_f value and identified by modern spectroscopic methods; FT IR, ^{1}H NMR, ^{13}C NMR and EI-MS.

Results and Discussion

In vitro antidiabetic Effect of Tubers of *D. bulbifera*

The treatment goal of diabetic is to maintain near normal level of glycemic control in both fasting and post-prandial conditions. Many natural sources have been investigated with respect to suppression of glucose production from the carbohydrates in the gut or glucose absorption from the intestine. α-amylase catalyses the hydrolysis of α-1,4-glucosidic linkages of starch, glycogen and various oligosaccharides to disaccharide and α-glucosidase further breaks down these disaccharides to simple sugars for intestinal absorption. The inhibition of their activities in the digestive tract is considered to be effective tool to control diabetes (Genapaty *et al.*, 2013).

α-amylase inhibitory activity

α-amylase inhibitory activity was measured *in vitro* by hydrolysis of starch in the presence of α-amylase enzyme. The amount of starch remained was determined by using iodine which gave blue colour with starch. The reduced intensity of blue colour indicated the enzyme-induced hydrolysis of starch. In the other words, the more the intensity of blue colour, the higher the inhibitory activity of the test sample on α-amylase enzyme. The extracts exhibited IC_{50} less than 100 μg/mL will be considered active in comparison with standard metformin and acarbose (Ganapaty *et al.,* 2013). Table 1 shows the inhibitory of α-amylase by the extracts of tubers of *D. bulbifera*. All test extracts showed dose dependent inhibitory of enzyme. The IC_{50} values of pet-ether, ethyl acetate, ethanol as well as standard metformin and acarbose were found to be 172.50, 62.50, 60.00 and 42.50 μg/mL respectively. Among these extracts, ethyl acetate extracts showed significant α-amylase inhibitory activity, its IC_{50} value (62.50 μg/mL) was higher than acarbose (IC_{50} 42.50) but nearly the same as metformin (IC_{50} 60.00 μg/mL).

Table 1 : α-Amylase Inhibition % and IC_{50} Values of Various Crude Extracts of Tubers of *D. bulbifera* Compared with Standard Metformin and Acarbose

Sample	% Inhibition (mean ± SD) in different concentrations (μg/mL)					IC_{50} (μg/mL)
	25	50	100	200	400	
PE (extract)	14.75± 0.69	24.35± 1.84	30.44± 1.06	55.82± 1.53	69.26± 1.76	172.50
EtOAc (extract)	34.10± 1.38	44.71± 0.89	72.59± 1.95	78.11± 0.91	84.26± 0.61	62.50
EtOH (extract)	15.65± 0.73	28.37± 0.89	43.02± 0.65	55.34± 1.17	61.81± 1.00	165.00
Metformin (Standard)	21.85± 0.73	45.30± 1.01	66.76± 1.07	75.09± 0.85	86.61± 0.90	60.00
Acarbose (Standard)	25.98 ± 0.66	60.32 ± 0.21	79.86±0. 73	85.96 ± 1.11	95.06 ± 0.89	42.50

Data are expressed as means of triplicate determination ± standard deviation.

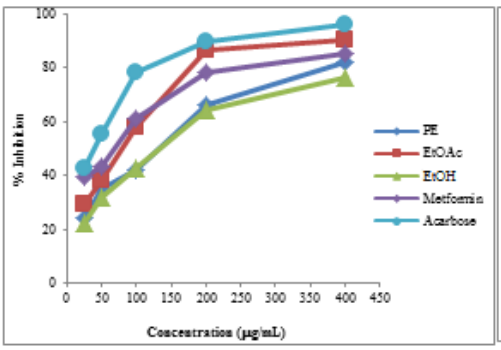


Figure 3: A plot of α-amylase inhibition activity and concentration of crude extracts of tubers of *D. bulbifera* compared with standard metformin and acarbose

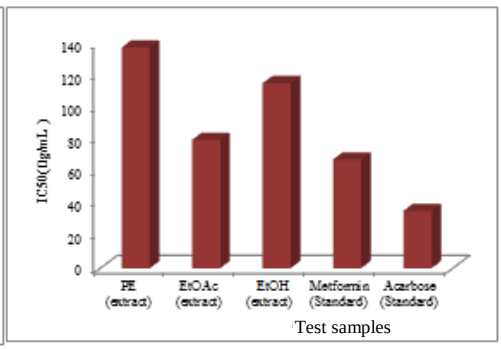


Figure 4: A bar graph of 50% α-amylase inhibitory concentration (IC_{50}) values of crude extracts of tubers of *D. bulbifera* compared with standard metformin and acarbose

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity was determined by glucose oxidase method in which the green Cu (II) complex of BCA was reduced to the violet Cu (I) complex by hydrogen peroxide (Warren *et al.,* 1990). Table 2 shows the α-glucosidase inhibitory activity of extracts of tubers of *D. bulbifera*. The order of α-glucosidase inhibitory activities were found to be acarbose (IC_{50} 35.75 μg/mL) > metformin (IC_{50} 62.75 μg/mL) > ethyl acetate (IC_{50} 80.00 μg/mL) > ethanol (IC_{50} 115.25 μg/mL) > pet ether (IC_{50} 137.50 μg/mL). So ethyl acetate showed potent α-glucosidase inhibitory activity than pet-ether and ethanol extracts.

Table 2: α-Glycosidase Inhibition % and IC_{50} Values of Various Crude Extracts of Tubers of *D. bulbifera* Compared with Standard Metformin and Acarbose

Sample	% Inhibition (mean ± SD) in different concentrations (μg/mL)					IC_{50} (μg/mL)
	25	50	100	200	400	
PE (extract)	23.94 ± 0.78	35.27 ± 0.59	42.15 ± 1.13	66.03 ± 0.88	82.16 ± 1.40	137.50
EtOAc (extract)	29.46 ± 1.98	38.27 ± 1.33	58.02 ± 1.63	86.46 ± 0.49	90.25 ± 1.05	80.00
EtOH (extract)	22.36 ± 0.25	31.99 ± 1.06	42.83 ± 0.53	64.12 ± 0.88	76.45 ± 0.35	115.25
Metformin (Standard)	39.54 ± 6.28	43.58 ± 2.50	61.44 ± 0.66	78.19 ± 0.25	85.54 ± 0.35	62.75
Acarbose (Standard)	42.83 ± 1.05	55.31 ± 0.49	78.07 ± 1.63	90.06 ± 1.33	96.37 ± 1.98	35.75

Data are expressed as means of triplicate determination ± standard deviation.

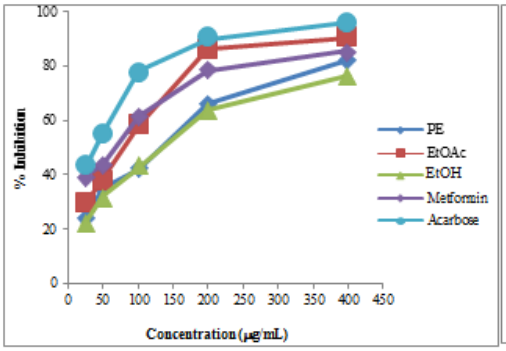


Figure 5:A plot of α-glucosidase inhibition activity and concentration of tubers of *D.bulbifera* compared with standard metformin and acarbose

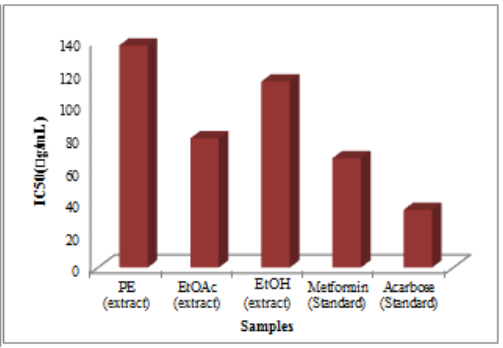
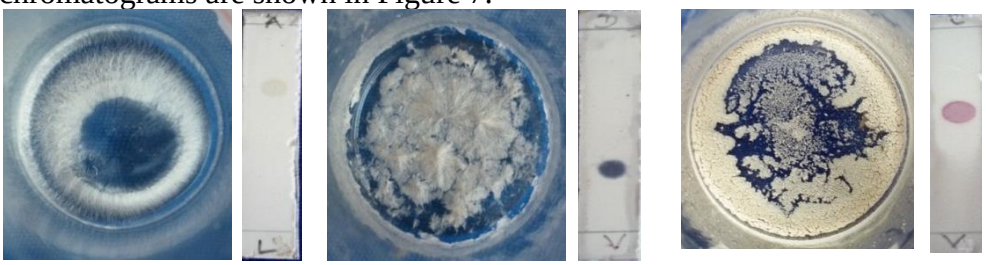


Figure 6:A bar graph of 50% α-glucosidase inhibitory concentration (IC_{50}) values of crude extracts of tubers of*D. bulbifera* compared with standard metformin and acarbose

Structure Elucidation of Isolated compounds

Three compounds; **A**, **B** and **C** were isolated from the active guided ethyl acetate extract by column chromatographic method and identified by spectroscopic methods. Photographs of isolated compounds and their TLC chromatograms are shown in Figure 7.



Compound A
Solvent = $CHCl_3$:MeOH, 19:1
Reagent = Liberman-Burchard,Δ
R_f= 0.72

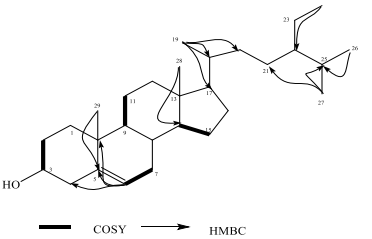
Compound B
Solvent = $CHCl_3$:MeOH, 19:1
Reagent = H_2SO_4 - Anisaldehyde,Δ
R_f= 0.33

Compound C
Solvent = $CHCl_3$:MeOH, 19:1
Reagent = H_2SO_4 - Anisaldehyde,Δ
R_f= 0.69

Figure 7: Photographs and thin layer chromatograms of isolated compounds **A**, **B** and **C** from EtOAc extract of tubers of *D. bulbifera*

Compound A **(β- sitosterol)**

Compound **A** was also isolated as a white powder and the molecular ion in EI-MS of **A** appearing at *m/z* 414 suggested the molecular formula as $C_{29}H_{50}O$. Compound **A** also showed positive Liebermann-Burchard reaction indicated sterol nature. The ^{1}H NMR spectrum of compound **A** showed the presence of six methyl signals that appeared as two methyl singlets at δ 0.68, and 1.01; three methyl doublets that appeared at δ 0.81, 0.83, and 0.93; a methyl triplet at δ 0.84; and one olefinic proton at δ 5.36. The ^{1}H NMR spectra of compound **A** showed a proton corresponding to the proton connected to the C-3 hydroxy group which appeared as a triplet of doublet of doublets at δ 3.53. The ^{13} C NMR together with COSY, HMQC and HMBC showed twenty nine carbon signal including six methyl, eleven methylene, ten methane and three quaternary carbons. Thus, the structure of **A** was assigned as β-sitosterol that was consistent to the reported literature values (Chaturvedula and Prakash, 2012) and was further supported by the key COSY and HMBC correlations as shown below. Some respective NMR spectra of **A** are shown in Figures 8, 9,b10 and 11.

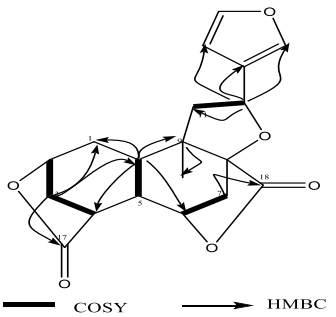


Key COSY and HMBC Correlation of compound A (β-sitosterol)

Compound B (Diosbulbin B)

Compound **B** was obtained as a white crystal. Its molecular formula was established as $C_{29}H_{20}O_6$ on the basis of EI-MS peak at *m/z* 344 (M^+ peak). Absorptions in the IR spectrum were attributable to *γ*- lactone (1773 and 1731 cm^{-1}) and a furan ring (1505 and 875 cm^{-1}).The ^{1}H, ^{13}C NMR and distortionaless enhancement by polarization transfer (DEPT) spectral data of **B** were the characteristic signals for a *β*- substituted furan ring (δ_C 145.0, 141.7, 126.6, and 109.7) and two *γ*- lactones (δ_C 176.9 and 178.8). The ^{1}H NMR spectrum, coupled with its ^{1}H-^{1}H correlation spectroscopy (^{1}H-^{1}H COSY)and heteronuclear single quantum coherence (HSQC) spectra, also

showed typical signals of a β-substituted furan ring at δ_H 7.62(1H, m, H-16),7.51(1H, m, H-15) and 6.55 (1H, m, H-14).The lactone carbonyl at δ_C 176.9 was assigned C-18 due to the HMBC correlations with proton signals at δ_H 1.75 (H-3) and 2.02 (H-5), and the correlation with oxymethine proton at δ_H 4.87 (H-2) indicated the lactone ring closure to C-2. Similarly the lactone carbonyl at δ_C 178.8 was assigned to C-17 due to the HMBC correlations with protons at δ_H 2.41 and 2.47 (H-7), while its ^{1}H-^{1}H COSY correlation with the proton at δ_H 4.79 (H-6) indicated the lactone ring closure to C-6. These NMR spectroscopic data of B similar to those of diosbulbin **B**, showing typical resonances for a norclerodanediterpenoid skeleton (Lin *et al.*, 2004) as below and some respective NMR spectra of **B** are shown in Figures 12, 13, 14 and 15.

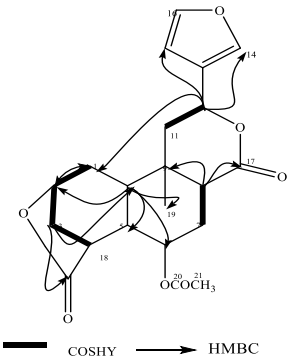


Key COSY and HMBC Correlation of Compound B (diosbulbin B)

Compound C

Compound **C** was obtained as a white amorphous with amolecular ion peak at *m*/*z* 388 in the EI-mass spectrum corresponding to molecular formula $C_{21}H_{22}O_7$.The^{13}C NMR analysis displayed 21 signals for all carbon atoms in the molecule, including three carbonyls, two non-protonated, ten methine, four methylene and two methyl carbons. The IR spectrum was consistent with the presence of the furan ring (1505 and 875 cm$-^1$),aγ-lactone(1781 and 1732 cm$-^1$). The absence of any vinyl proton resonance in the ^{1}H NMR spectrum, apart from the characteristic signals for a β- substituted furan ring, required the compound that contained one tertiary methyl group to be bicyclic. The γ-lactone ring was fused to ring A, the methane proton appeared at δ_H 4.88 (H-2) and showing coupling interactions with δ_H 1.88 (H-1), δ_H 2.53 (H-3)

and δ_H 2.60 (H-4) signals in its ^{1}H- ^{1}H COSY correlation. From the combination of ^{1}H- ^{1}H COSY, HSQC and HMBC, the structure of **C**was assigned as 8-epidiosbulbin E acetate that was consistent to the reported literature values(Shriram*et al.*, 2008). Some respective NMR spectra of **C** are shown in Figures 16, 17, 18 and 19.



Key COSY and HMBC Correlation of Compound C (8-epidiosbulbin E acetate)

β-sitosterol (A): white crystal, m.p 139 °C, R_f 0.72 ($CHCl_3$:MeOH ,19:1 v/v), FT IR (υ_{max} cm^{-1}):3440 (υ_{OH}), 2935, 2867 (υ_{C-H}asym and sym), 1637 ($\upsilon_{C=C}$), 1465 (δ_{C-H}), 1063 (υ_{C-O}) and 958 ($\delta_{oop(C-H)}$), ^{1}H NMR (600 Hz, $CDCl_3$), δ_H : 0.68 (s, 3H, H-28), 0.81 (d, 3H, *J* = 6.4 Hz, H-27), 0.83 (d, 3H, *J* = 6.4 Hz, H-26), 0.93 (d, 3H, *J* = 6.5 Hz, H-19) 0.84 (t, 3H, *J* = 7.2 Hz, H-24),1.01 (s, 3H, H-29), 3.53 (tdd, 1H, *J* = 4.5, 4.2, 3.8 Hz. H-3),5.36 (t, 1H, *J* = 6.4 Hz. H-5),^{13}C NMR (150 Hz, $CDCl_3$), δ_C : 19.2 (C-19), 12.2 (C-24), 20.1 (C-26), 19.6 (C-27), 19.0 (C-28), 12.1(C-29), 37.5 (C-1), 31.9 (C-2), 42.5 (C-4), 32.1 (C-7), 21.3 (C-11), 40.0 (C-12), 24.5 (C-15), 28.5 (C-16), 34.2 (C-20), 26.3 (C-21), 23.3 (C-23), 72.0 (C-3), 121.9 (C-6), 32.1 (C-8), 50.4 (C-9), 57.0 (C-14), 56.3 (C-17), 36.3 (C-18), 46.1 (C-22), 29.9 (C-25), 140.9 (C-5), 36.7 (C-10), 42.5 (C-13). EI-MS m/z: 414 [M^+], 396, 381, 329, 303, 273, 255, 213, 159, 145, 95, 81, 55.

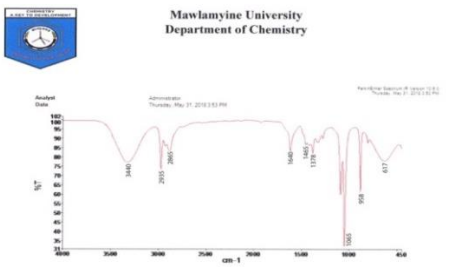


Figure 8: FT IR spectrum of the isolatedcompound **A**

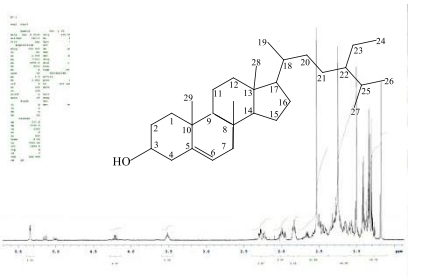


Figure 9: ^{1}H NMR spectrum of the isolated compound **A** (600 MHz, $CDCl_3$)

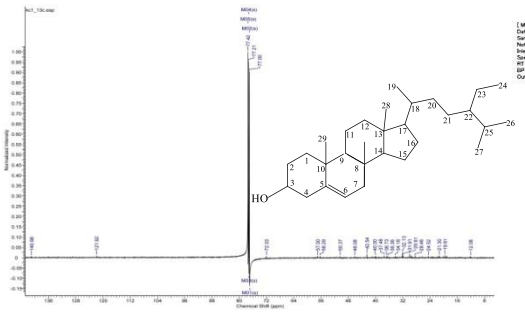


Figure 10: ^{13}C NMR spectrum of the isolated compound **A**(150 MHz, $CDCl_3$)

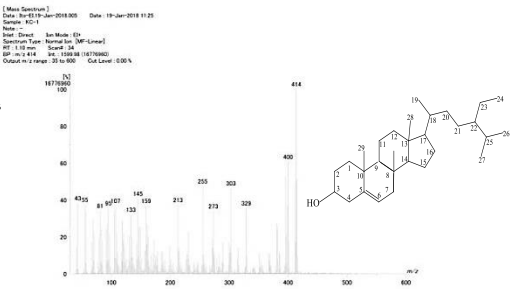


Figure11:EI-mass spectrum of the isolated compound **A**

Diosbulbin B (B): white crystal, m.p 217 °C, R_f0.33 ($CHCl_3$:MeOH, 19:1 v/v), FT IR (υ_{max} cm^{-1}); 2983, 2885 (υ_{CH}), 1773, 1731 ($\upsilon_{C=O}$), 1505 ($\upsilon_{C=C}$), 1463, 1371 (δ_{C-H}), 1252 (υ_{C-O}) and 875 ($\delta_{oop(C-H)}$), ^{1}H NMR (600 Hz, $CDCl_3$), δ_H: 7.66 (1H, s, H-16), 7.57 (1H, d, *J* = 1.8 Hz, H-15), 6.85 (1H, d, *J* = 1.8 Hz, H-14), 1.7 (3H, s, H-19), 4.84 (1H, ddd, *J* = 5.0, 5.5, 0.8 Hz, H-2), 4.76 (1H, d, *J* = 5.5 Hz, H-6), 5.24 (1H, dd, *J* = 5.0, 5.5 Hz, H-12), 2.71 (1H, d, *J* = 5.5 Hz, H-4), 2.16 (1H, d, *J* = 5.5 Hz,H-5), 1.63 (1H, m, H-10), 1.63 (H-1β), 1.86 (H-1α), 1.90 (H-3β), 2.38 (H-3α), 2.13 (H-7β), 2.43 (H-7α), 1.80 (H-11β), 2.00 (H-11α),^{13}C NMR (150 Hz, $CDCl_3$), δ_C: 176.1 (C-17) and 177.3 (C-18), 143.1 (C-15), 140.9 (C-16), 125.9 (C-13), 110.2 (C-14), 76.6 (C-2) 77.1 (C-6), 74.2 (C-12), 89.3 (C-8), 45.3 (C-9), 28.7 (C-1), 38.2 (C-3), 36.7 (C-7) and

41.5 (C-11), 40.9 (C-5), 41.3 (C-4), 38.5 (C-10), 46.1 (C-19). EI-MS m/z: 344 [M^+], 300 [$M^+ - CO_2$], 255 [M^+ m/z 300 – COOH], 206 [$M^+ - C_7H_6O_3$].

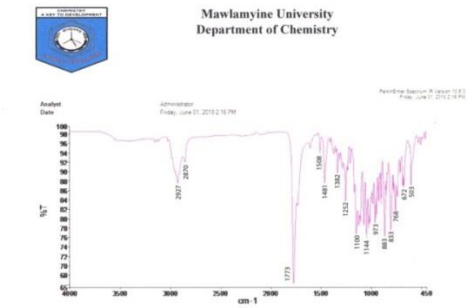


Figure 12:FT IR spectrum of the isolated compound **B**

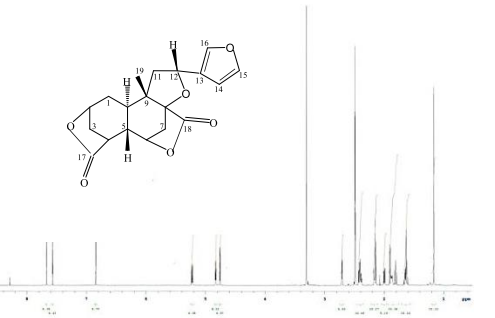


Figure 13:^{1}H NMR spectrum of the isolated compound **B** (600 MHz, $CDCl_3$)

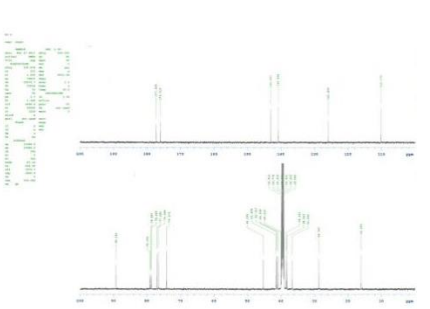


Figure 14:^{13}C NMR spectrum of the isolated compound **B** (150MHz, $CDCl_3$)

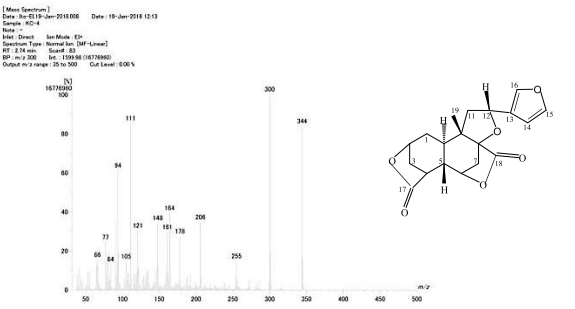


Figure 15:EI-mass spectrum of the isolated compound **B**

8-Epidiosbulbin E-acetate (C): white amorphous, m.p 255 °C, R_f0.69 ($CHCl_3$:MeOH, 19:1 v/v), FT IR ($\upsilon_{max}cm^{-1}$): 2962-2860 (υ_{CH}), 1783, 1769, 1701 ($\upsilon_{C=O}$), 1509 ($\upsilon_{C=C}$), 1460, 1330 (δ_{C-H}), 1252 (υ_{C-O}), 876 ($\delta_{oop(C-H)}$),^{1}H NMR (600 Hz, DMSO), δ_H: 7.66 (1H, s, H-14), 6.45 (1H, s, H-15), 7.70 (1H, s, H-16), 4.80 (1H, m, H-2), 4.75 (1H, dd, *J* = 5.5, 16.3 Hz, H-6), 2.60 (1H, m, H-4), 1.90 (1H, m, H-5), 3.05 (1H, m, H-8), 2.65 (1H, m, H-10), 1.88 (H-1α), 1.63 (H-1β), 2.53 (H-3α), 1.75 (H-3β), 3.01 (H-7α), 1.99 (H-7β), 2.14 (H-11α), 1.78 (H-11β), 1.16 (3H, s, H-19), 1.99 (3H, s, H-21),^{13}C NMR (150 Hz,

DMSO),δ_C: 170.1 (C-20), 174.1 (C-17), 176.7 (C-18), 109.2 (C-14), 124.6 (C-13), 140.1 (C-15), 143.9 (C-16), 69.4 (C-12), 69.5 (C-6), 76.7 (C-2), 36.7 (C-4), 41.2 (C-5), 41.6 (C-8), 41.6 (C-10), 27.7 (C-7), 28.8 (C-1), 38.1 (C-3), 41.1 (C-11), 16.2 (C-19), 21.0 (C-21), 35.5 (C-9). EI-MS m/z: 388 [M^+], 346 [M^+–(–CH_2=C=O)], 328 [M^+– $C_2H_4O_2$], 43 [M^+– $C_{19}H_{21}O_6$].

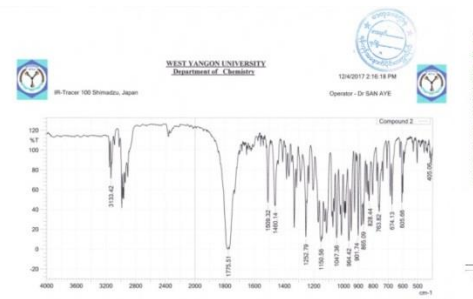


Figure 16:FT IR spectrum of the isolated compound **C**

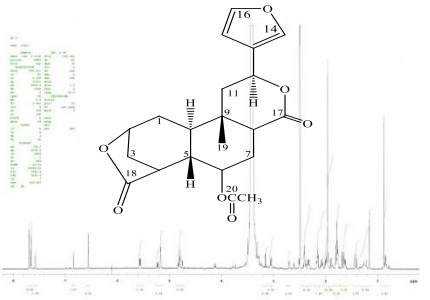


Figure 17:^{1}H NMR spectrum of the isolated compound **C** (600 MHz, DMSO)

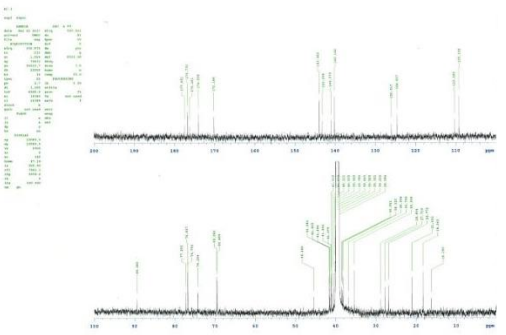


Figure 18:^{13}C NMR spectrum of the isolated compound **C** (150 MHz, DMSO)

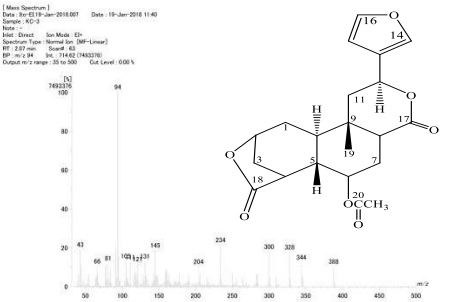


Figure 19:EI-mass spectrum of the isolated compound **C**

Conclusion

The present study deals with the investigation of antidiabetic activity of tubers of *D. bulbifera* and isolation of some phytoconstituents from it. *Invitro* α-amylase inhibitory activity was determined by starch iodine method and the resultant activities were compared with standard antidiabetic drugs; acarbose and metformin. The order of α-amylase inhibitory activities of crude

extracts were found as:acarbose(IC_{50} 42.50 μg/mL) > Metformin (IC_{50} 60.50 μg/mL) >EtOAc (IC_{50} 62.50 μg/mL)>MeOH (IC_{50} 165.00 μg/mL) > PE (IC_{50} 172.50 μg/mL).*In vitro* α-glucosidase inhibitory of crude extracts of tubers of *D. bulbifera*and standard acarbose and metformin were measured by glucose oxidase method. The IC_{50} values of acarbose, metformin, PE, EtOAc and MeOH extracts were found to be 35.75, 67.75, 137.50, 80.00 and 115.25 *m*g/mL respectively. These test results revealed that ethyl acetate extract showed potent inhibitory effect on both α-amylase and α-glucosidase enzymes.

Compounds **A**, **B** and **C** were isolated from activity guided ethyl acetate fraction by column chromatography and characterized by FT IR, ^{1}H NMR, ^{13}C NMR and EI-MS specroscopic methods. These isolated compounds were identified as β-sitostoral (**A**) (0.0412 % yield, m.p 139 °C), diosbulbin **B** (**B**) (0.002 % yield, m.p 217 °C) and 8-epidiosbulbin E-acetate (**C**) (0.0210 % yield, m.p 225 °C).

It is evident that ethyl acetate extract of tubers of *D. bulbifera* has significant antidiabetic effect and it is likely due to the presence of bioactive phytoconstituents, such as steroids and terpenoids. So, it can be inferred that tubers of *D. bulbifera* have invaluable medicinal use for the treatment of hyperglycemia in diabetes.

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