



Membrane stabilization and probable mechanisms of hypoglycemic activity of fruit extract of *Solanum incanum* L. (Solanaceae)

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Abstract

This study investigated the antioxidant, membrane stabilization, and mechanisms of hypoglycemic attribute of aqueous fruit extract of *Solanum incanum* L. in vitro. The extract was separately incubated with α -amylase and α -glucosidase and subsequently with starch and *p*-nitrophenylglucopyranoside, respectively, while its glucose uptake regulatory effect was evaluated against *Saccharomyces cerevisiae*. The data obtained showed that the extract significantly modulated the rate of glucose uptake by yeast cells and had respective competitive and uncompetitive inhibitory influence on α -glucosidase and α -amylase with overall half-maximal values of 2.98 and 7.08 mg mL⁻¹ relative to that of acarbose (4.93 and 5.59 mg mL⁻¹). *S. incanum* also markedly halts free radicals in a manner comparable to silymarin. With a display of 86.67% potency against heat-induced hemolysis of bovine serum erythrocytes, the extract could also be said to have shown marked membrane stabilization activity. The effects shown by *S. incanum* at the investigated concentrations may be ascribed to its analytes as revealed by the results of the chromatographic analysis. Put together, besides being antioxidative, regulation of glucose uptake rate and enzymes' activities are its probable mechanisms of hypoglycemic potential and has further provided baseline evidence for its antidiabetic application.

Keywords Acarbose · Antidiabetic · Competitive inhibition · Hypoglycemic · *S. incanum* · Yeast cell

Introduction

The global upsurge in the prevalence of diabetes mellitus (DM) has remained a key health threat with significant economic burden (Bakirel et al. 2008). While DM is on the increase in the developing countries, it has assumed an epidemic

state in industrialized nations, with the type 2 form accounting for over 90% of reported cases (Abdulazeez and Ponnusam 2016). With this report, more than 12% (727 billion dollars) of the global health expenditure is currently spent on the disease and about 552 million people of the world's population are estimated to be diabetic by the year 2030, if no practical solution is sought (IDF 2017).

Besides lifestyle abuses, free radicals and reactive oxygen species-mediated oxidative stress are also fundamental to the pathogenesis of DM (Sabiu and Ashafa 2016). Although the conventional oral hypoglycemic drugs have been effective in the treatment and management of DM, high cost and significant adverse effects have undermined their usage. Little wonder, new and emerging evidence-based phytotherapeutic studies geared towards diabetes management are now exploring more affordable and easily accessible natural plant formulations endowed with reasonable antioxidant attributes (Gull et al. 2011; Ghosh et al. 2013). Such studies are not only envisaged to pave ways for a new therapeutic strategy for diabetes treatment and its related complications (Mendes et al. 2015; Sabiu et al. 2017) but would also offer safer and less expensive adaptogenic biological agents that would ultimately improve the quality of life of diabetic patients (Ray et al. 2016). This is the case with the Solanaceae family, with

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most of its members potentiating significant anti-hyperglycaemic activity (Gupta et al. 2005; Poongothai et al. 2011).

Solanum incanum L (SI) also called sodom/bitter apple/garden egg belongs to the family Solanaceae (Schmelzer and Gurib-Fakim 2008). It is an erect or spreading perennial herb/shrub of about 1.8 m, with occasional spines on the leaves and stems, and velvet haired leaves (Sambo et al. 2000). The berry fruits are about 2–3 cm in diameter and are initially yellowish but later turn black (Matu 2008; Sambo et al. 2016). SI grows freely in the Middle and Far East Asia and many regions of Africa including Nigeria (Mwonjoria et al. 2014). Its therapeutic significance as anti-inflammatory, anti-cancer, antimicrobial, antinociceptive, antipyretic, and spasmolytic agents have been documented (Assefa et al. 2006; Taye et al. 2011; Alamri and Moustafa 2012). It is an ethnomedicinally valued vegetable for treating several ailments including diabetes. Although its fruit extracts have been studied to elicit good in vivo hypoglycemic potential (Musabayane et al. 2006; Uchenna et al. 2009), information on its tentative mechanism is lacking till date. Hence, this study investigated its probable mechanisms of hypoglycemic activity against *Saccharomyces cerevisiae* glucose uptake and the activities of prominent enzymes (α -glucosidase and α -amylase) linked to carbohydrate metabolism. The study also evaluated its chemical profiling, membrane stabilization, and antioxidant potentials.

Methodology

Sample collection and authentication

Healthy fruits of *Solanum incanum* were purchased from a local vendor at Ilorin, Nigeria. They were identified at the Herbarium unit, University of Ilorin, Nigeria, by Mr. Bolu Ajayi, and a reference number (UILH/002/1045) was issued for its voucher specimen that was subsequently deposited for future reference.

Extract preparation

The fruits of SI were thoroughly washed with distilled H₂O, chopped, and air-dried to constant weight. This was then powdered and a portion (100 g) of it was suspended in 1000 mL of distilled H₂O for 24 h under constant agitation. Using Whatman No. 1 filter paper, the resulting mixture was filtered and subsequently lyophilized (40 °C). The extract obtained (SIAE) was kept airtight and refrigerated before use.

Reagents and chemicals

The 1,1-diphenyl-2-picrylhydrazyl (DPPH), *Saccharomyces cerevisiae* (yeast), acarbose, porcine pancreatic α -amylase, rat intestine acetone powder (RIAP), and para-nitrophenyl-

glucopyranoside (pNPG) were obtained from Sigma-Aldrich, USA. Soluble starch (extra pure) and dinitrosalicylic acid (DNS) were products of J. T. Baker Inc., USA, while other reagents and chemicals used were of good analytical standard.

In vitro radical scavenging assays

DPPH assay

In this assay, a previously reported method (Turkoglu et al. 2007) was adopted in evaluating the capability of SIAE to scavenge DPPH free radicals using silymarin as standard. The inhibitory ability (I%) of SIAE on DPPH radical was then calculated and the concentration of SIAE produced 50% inhibitory (IC₅₀) effect extrapolated from a standard curve.

Hydrogen peroxide and hydroxyl radical (OH[•]) inhibitory potential

The reported procedures of Ruch et al. (1989), and Smirnoff and Cumbes (1996) were respectively used for the determination of the H₂O₂ and OH[•] radicals-scavenging potential of SIAE. While silymarin served as standard, sterile distilled H₂O₂ replaced SIAE and served as control in each case. The IC₅₀ values were also calculated from the calibration graph.

Lipid peroxidation assay

In this experiment, the capability of SIAE to halt generation of lipid peroxide in an egg yolk homogenate as described by Oyedemi et al. (2012) was adapted. Distilled H₂O was used as blank and silymarin as reference standard.

Membrane stabilization activity

Bovine erythrocyte suspension preparation and heat-induced hemolysis assay

A modified procedure of Oyedapo et al. (2010) was employed in this assay. Bovine whole blood was taken in EDTA-coated tubes and centrifuged (10 min, 3000 rpm). The plasma and leucocytes (supernatant) was removed, while the pellet (packed erythrocytes) was carefully rinsed severally with a solution containing NaCl (154 mM) prepared in sodium phosphate buffer (10 mM, pH 7.4) and recentrifuged (10 min, 3000 rpm) to have a clear supernatant. Subsequently, the final pellet obtained was used in the preparation of the stock suspension (2% v/v) of red blood cells (erythrocytes) used in this assay.

For the assay, an isotonic buffer (4.5 mL) containing either SIAE (0.25–2.0 mg mL⁻¹; 0.5 mL) or ibuprofen (0.1 mg mL⁻¹; 0.5 mL) was put into two sets of centrifuge tubes. For the control, 5.0 mL of distilled H₂O was kept in another tube. Thereafter, 0.5 mL of the erythrocyte stock suspension was introduced into

each tube and vortexed by inversion. While a set of the tube was maintained on ice bath (0–5 °C), the second set was kept in a water bath (54 °C, 20 min). Following this, each set up was centrifuged (5 min, 3000 rpm,) prior to spectrophotometric absorbance reading (540 nm) of the hemoglobin liberated into the supernatant. The % protection conferred by SIAE against hemolysis of the erythrocyte in the test sample was thereafter evaluated from the expression:

%protection offered on erythrocyte against hemolysis

$$= (A_H - A_U / A_C - A_U) \times 100.$$

A_C , A_H , and A_U represent the respective absorbance readings of the heated sample (control), heated test sample, and unheated test sample.

Hypoglycemic studies

In vitro glucose uptake modulatory assay

The *S. cerevisiae* used in this study was made in accordance with an earlier reported procedure (Gupta et al. 2012). Briefly, 2 g of *S. cerevisiae* was continuously rinsed under centrifugation (4200 rpm) in sterile distilled H₂O to have a reasonably clear supernatant. Thereafter, a suspension of the yeast cell (10% v/v) was prepared in distilled H₂O to serve as stock. Various concentrations (1.0–5.0 mg) of SIAE were mixed with 2 mL each of glucose solutions (5.0–25.0 mmol/L) in different tubes prior to incubation (10 min, 37 °C). Following this, 200 µL of the yeast suspension was mixed with the resulting content in the tube for reaction initiation. This was then thoroughly mixed and incubated (60 min, 37 °C). Finally, the reaction mixtures were centrifuged (5 min, 3800 rpm) and the supernatant obtained in each case was spectrophotometrically measured (515 nm) as the concentration of glucose in each tube. The increase in the uptake of glucose by *S. cerevisiae* at each SIAE concentration was then evaluated from the expression:

%Increase in uptake of glucose

$$= 100 \times [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}].$$

Abs_{sample} and Abs_{control} respectively represent the absorbance of the sample (test) and that of the control (comprising all the reagents save the sample (test)).

Carbohydrate-metabolizing enzymes' inhibitory studies

Alpha-amylase inhibitory assay

This involves preparation of stock solutions of 0.25–10.0 mg/mL of either SIAE or acarbose (standard) in distilled H₂O.

From the stocks, 500 µL each was added to 500 µL of 0.5 mg/mL of ice-cold solution of porcine pancreatic α -amylase in sodium phosphate buffer (0.02 M, pH 6.9) and incubated (10 min, 25 °C). Subsequently, 500 µL of starch (1%) solution in sodium phosphate buffer (0.02 M, pH 6.9) was introduced prior to further incubation (10 min, 25 °C). DNS (1.0 mL) was added to halt the reaction and the content was heated (5 min, 100 °C), completely cooled (25 °C) and 15.0 mL of sterile distilled H₂O was added prior to absorbance reading (540 nm). The control contained the buffer (500 µL) instead of SIAE and the experiments were replicated three times. The inhibitory potential of SIAE on alpha-amylase was then calculated and presented as % from:

Inhibitory effect (%) = 100

$$\times [(\Delta A_{\text{control}} - \Delta A_{\text{extract}}) / \Delta A_{\text{control}}].$$

$\Delta A_{\text{extract}}$ and $\Delta A_{\text{control}}$ are the respective changes in absorbance of the SIAE and control. The IC₅₀ value of SIAE against alpha-amylase was thereafter calculated using calibration plot (Mohamed et al. 2012).

To determine the kinetics parameters of the inhibitory effect of SIAE against the activity of α -amylase, 100 µL of either the extract (at its IC₅₀) or sodium phosphate buffer (0.02 M, pH 6.9) was incubated (10 min, 25 °C) with α -amylase solution (100 µL) in two sets of test tubes. Following this, starch (0.3–5.0 mg/mL) corresponding to substrate (*S*) was introduced into both sets of tubes for the reaction to start. Finally, the resulting solution in each case was further treated with DNS (100 µL), boiled (5 min, 100 °C), cooled (25 °C) and diluted with sterile distilled H₂O. The absorbance of the released sugar was then taken (540 nm), values estimated from maltose standard curve and subsequently expressed as reaction rates (*v*). The kinetic indices (K_m and V_{max} values) and the tentative mechanism of inhibitory action of SIAE on alpha-amylase activity were then deduced (Lineweaver and Burk 1934).

Alpha-glucosidase inhibitory assay

The solution of alpha-glucosidase used was prepared as earlier reported (Oki et al. 1999). This was done by homogenizing 25.0 mg/mL of RIAP in ice-cold phosphate buffer (50 mM) followed by centrifugation (6000×g, 15 min). The resulting clear supernatant represents alpha-glucosidase solution. For the assay, the procedure of Elsnoussi et al. (2012) was used. Briefly, 0.25–10.0 mg/mL of either SIAE or acarbose were prepared and 50 µL from each stock solution was introduced into 1.0 M of alpha-glucosidase solution (100 µL) prepared in phosphate buffer (0.1 M, pH 6.9). This was then incubated (10 min, 25 °C) in 96-well plates prior to the addition of 50 µL of 5 mM *p*NPG solution (made in phosphate buffer (0.1 M, pH 6.9)). The resulting solutions were further incubated

(5 min, 25 °C) and the absorbance in each case was read (405 nm) in a micro-plate reader. The values obtained were compared with that of the control which had 50 µL of the buffer in place of SIAE. The inhibitory potential of SIAE on the alpha-glucosidase activity was calculated and the IC₅₀ was subsequently determined from the standard calibration curve.

To further determine the inhibitory kinetics of SIAE on activity of α-glucosidase, 50 µL of either SIAE at its IC₅₀ value or phosphate buffer (pH 6.9) was pre-incubated (10 min, 25 °C) with solutions of alpha-glucosidase (100 µL) in two sets of tubes. Subsequently, 50 µL each of 0.63–2.0 mg/mL (*p*NPG) was introduced into the two set-ups for reaction initiation before allowing an incubation period of 10 min at 25 °C. To halt the reaction, Na₂CO₃ (500 µL) was added and the released sugar in each case was colorimetrically estimated from para-nitrophenol calibration curve. The reaction rates (*v*) were then estimated and Lineweaver-Burk double reciprocal plot was used to predict the nature of inhibition of the enzyme by SIAE.

Phytoconstituent analysis of SIAE using GC-MS

The Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film) was used in the analysis. The concentration of the individual constituent in SIAE was presented as % on the basis of the peak area in the chromatogram. For their identification, matching of their characteristic features with the different components in the NIST library was used.

Statistical evaluation

Except otherwise stated, data were processed by one-way analysis of variance complemented with Duncan's Multiple Range Test using SPSS software package for windows V16.0. Results are represented as mean ± standard error of the mean (SEM) of triplicate determinations. At a *p* < 0.05 confidence level, the values of the mean were considered statistically significant.

Results

Membrane stabilization and antioxidant activities of *S. incanum* fruit aqueous extract

The in vitro antioxidant potential of SIAE and the respective coefficient of determination (*R*²) values are presented in Table 1. While the extract compared favorably well with the standard (silymarin) at scavenging DPPH, OH[−] and LPO[−] radicals, it significantly (*p* < 0.05) exhibited a potent H₂O₂ scavenging activity than silymarin (Table 1).

The data obtained with respect to the membrane stabilization potential of SIAE showed that it was concentration-dependently effective in conferring protection on bovine RBC against heat-induced hemolysis. The observed effect at 2 mg/mL dose compared well with ibuprofen (Table 2).

Mechanisms of hypoglycemic potential of SIAE

The glucose uptake modulatory activity of SIAE on *S. cerevisiae* revealed a concentration-dependent effect and reduced as glucose concentration increases (Fig. 1). At 5 mg SIAE, maximum activity was observed across all the glucose concentrations with the most prominent effect (89.35%) elicited in the presence of 5 mM glucose treatment (Fig. 1).

Table 3 shows the inhibitory potential of SIAE on the activities of alpha-glucosidase and alpha-amylase. When compared with SIAE (IC₅₀: 7.08 mg/mL), the standard (acarbose) elicited the best inhibitory ability (IC₅₀: 5.59 mg/mL) against alpha-amylase activity. A probe into the elucidation of its kinetics showed a reduction in both the values of *K_m* and *V_{max}* relative to the control (Fig. 2). The respective values for the *V_{max}* and *K_m* for the control were 0.04 µM/min and 0.0012 mg as against 0.02 µM/min and 0.0006 mg for SIAE, thus indicating an uncompetitive inhibitory action (Fig. 2). On the contrary however, SIAE had the most potent (IC₅₀: 2.98 mg/mL) inhibitory effect (*p* < 0.05) on alpha-glucosidase activity relative to that of acarbose (IC₅₀: 4.93 mg/mL) (Table 3) and further investigation into its mode of inhibitory influence on the enzyme revealed a competitive

Table 1 Antioxidant properties of *S. incanum* aqueous fruit extract

Treatment	DPPH		H ₂ O ₂		OH [−]		LPO [−]	
	IC ₅₀ (mg.mL ^{−1})	R ²	IC ₅₀ (mg.mL ^{−1})	R ²	IC ₅₀ (mg.mL ^{−1})	R ²	IC ₅₀ (mg.mL ^{−1})	R ²
SIAE	0.55 ± 0.02	0.8998	0.45 ± 0.01*	0.9543	0.34 ± 0.01	0.9973	0.28 ± 0.03	0.9765
Silymarin	0.52 ± 0.03	0.9193	0.89 ± 0.02	0.8978	0.37 ± 0.05	0.9456	0.30 ± 0.02	0.9678

*Significantly different from silymarin (*p* < 0.05) for the assay. SIAE = *Solanum incanum* aqueous extract

Table 2 Membrane stabilization effect of *S. incanum* aqueous fruit extract (SIAE)

Parameters	SIAE (mg.mL ⁻¹)						
	Control	Ibuprofen	0.25	0.50	1.00	1.50	2.00
A _{HS}	0.92 ± 0.03 ^a	0.86 ± 0.03 ^b	0.35 ± 0.02 ^c	0.46 ± 0.02 ^d	0.51 ± 0.01 ^e	0.69 ± 0.03 ^f	0.88 ± 0.01 ^{ab}
A _{US}	0.59 ± 0.04 ^a	0.44 ± 0.04 ^b	0.19 ± 0.01 ^c	0.28 ± 0.02 ^d	0.32 ± 0.04 ^d	0.40 ± 0.02 ^d	0.62 ± 0.02 ^a
Protection (%)	–	87.50	21.92	28.13	31.67	55.77	86.67

^{abcdef} Values not bearing the same letters in the same row for the parameter are different significantly at $p < 0.05$. A_{US}; absorbance of unheated sample, A_{HS}; absorbance of heated sample

type of inhibition (Fig. 3). By this, the $V_{\max}$ value (0.03 $\mu\text{M}/\text{min}$) remained constant for both the control and SIAE, while the K_m values increased from 0.001 mg for the control to 0.002 mg for the extract (Fig. 3).

Compounds belonging to flavonoids (kaempferol, quercetine), carotenoids (lycopene), alkaloids (nicotine, scopolamine, atropine), and saponin (solanin, solanine, solanidine, agamenoside A) were identified as the main constituents of SIAE as revealed from the results of GC/MS analysis conducted (Fig. 4 and Table 4).

Discussion

The global efforts on the search for novel therapeutic agents for the management of DM have advanced relentlessly. A good number of such efforts have been on the use of herbal medicines with significant hypoglycemic effect (Musabayane et al. 2006; Sabiu et al. 2017). Recently, research have not only been conducted on the antidiabetic effect of plants using different experimental models, but further investigations into their tentative mechanism(s) of action have been in the forefront (Gupta et al. 2012; Sabiu and Ashafa 2016). For instance, the probing into the manner of glucose transport across the membrane of *S. cerevisiae* has been receiving attention as a reliable and suitable in vitro method to evaluate

hypoglycemic effect of taxa. The transport of glucose across the membrane of *S. cerevisiae* is extremely complex and facilitated by diffusion. However, the effective and efficient removal of intracellular glucose by a suitable agent makes it a coveted model of interest and the glucose concentration left in the system after a particular period is indicative of the glucose uptake by *S. cerevisiae* (Teusink et al. 1998). In this study, the observed dose-dependent increase in the % inhibition of glucose uptake in *S. cerevisiae* subsequent to the increased concentration of SIAE is a probable indication of the hypoglycemic potential of the extract. This may also imply that the extract is endowed with phytoconstituents that could either effectively modulate or regulate removal of intracellular glucose which is a key regulatory mechanism in diabetes. The effect elicited by SIAE in this respect agrees with earlier reports (Gupta et al. 2012; Karpagasundari and Kulothungan 2015), where the authors demonstrated the respective significant glucose uptake inhibitory potential of the extracts of *Bauhinia purpurea* Linn and *Physalis minima* on yeast cells.

Similarly, of the carbohydrates-metabolizing enzymes, alpha-amylase and alpha-glucosidase represent the main pharmacological targets in the management of DM (Krentz and Bailey 2005). These enzymes facilitate hydrolysis of starch to glucose with consequential increase in the systemic concentration of glucose in diabetes. This increased hyperglycemia may constitute a significant risk factor for complications

Fig. 1 Glucose uptake modulatory effect of aqueous fruit extract of *S. incanum* (SIAE) on *Saccharomyces cerevisiae*

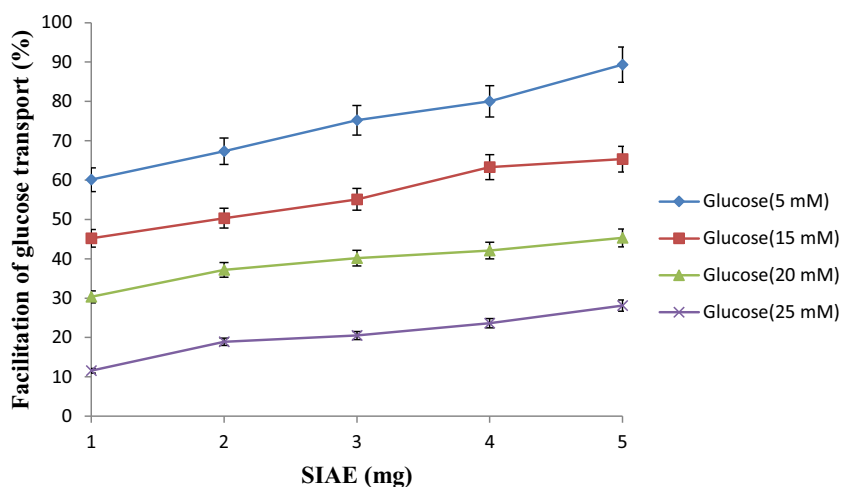


Table 3 Inhibitory activity of *S. incanum* fruit extract on the specific activities of alpha-glucosidase and alpha-amylase

Concentration (mg mL ⁻¹)	Inhibitory effect (%)			
	Alpha-amylase		Alpha-glucosidase	
	Acarbose	Extract	Acarbose	Extract
0.25	10.25 ± 0.03	8.36 ± 0.02	15.73 ± 0.09	25.01 ± 0.11*
0.50	15.17 ± 0.01	17.52 ± 0.09	25.89 ± 0.10	39.68 ± 0.09*
2.50	30.89 ± 0.04	25.32 ± 0.01*	40.11 ± 0.07	52.81 ± 0.01*
5.00	53.68 ± 0.02	52.11 ± 0.10	60.59 ± 0.08	61.92 ± 0.08
10.0	75.39 ± 0.09	58.95 ± 0.08*	72.19 ± 0.10	90.55 ± 0.05*
IC ₅₀	5.59	7.08*	4.93	2.98*
	(R ² = 0.9684)	(R ² = 0.8912)	(R ² = 0.8996)	(R ² = 0.9469)

(n = 3, mean ± SEM). *Different significantly (p < 0.05) from the respective reference (acarbose)

associated with oxidative stress-mediated DM (Kim et al. 2000). Hence, targeting to inhibit the specific activities of these enzymes has been identified as another viable mechanism to delay glucose absorption and modulate hyperglycemia (Sabiou et al. 2016). Conventionally, the use of oral hypoglycemic agents have been the therapeutic measure employed to lower blood glucose level, however, the associated unpleasant consequences have undermined their application (Sabiou and Ashafa 2016). Conversely, emerging findings suggest a shift to the development of safer and more effective antidiabetic herbal remedies with lesser side effects (Elsnoussi et al. 2012; Sabiou et al. 2017). In this study, judging by the IC₅₀ values of SIAE relative to that of the acarbose, it may be logically inferred that the extract potentiated milder action on alpha-amylase activity than alpha-glucosidase and this has been therapeutically identified to be significant in annihilating the adverse effects consistent with the conventional inhibitors of the two enzymes (Shai et al. 2010). This observation implies that the extract is a probable pharmacologically potent inhibitor of carbohydrate metabolizing enzymes and agrees with previous submission (Kwon et al. 2008). The reduction in the values of K_m and V_{max} for the extract (predicting its uncompetitive modulatory action on the

activity of alpha-amylase) may suggest a higher affinity of the enzyme for SIAE than the substrate, thereby strategically regulating further hydrolysis of systemic carbohydrate. Furthermore, the competitively inhibited activity of the α -glucosidase is indicative of a stronger structural resemblance of the major constituent of the extract to that of the substrate. This favorably facilitated the binding of the extract on the enzyme's active site at the expense of the substrate. As a result, the rate of starch hydrolysis to free glucose is either effectively reduced or abolished.

For decades, the relationship between free radicals generation and the pathogenesis of DM and its associated risk disorders has been established (Scartezzini and Speroni 2000; Modak et al. 2007). In the present study, SIAE elicited marked antioxidant activities as evident from its IC₅₀ values when compared with those of silymarin. This observation may be suggestive of the tendency of the extract to either regulate or stall free radicals chain reactions associated with diabetes complications. Apart from being consistent with previous in vitro studies (Sabu and Kuttan 2002; Sabiou et al. 2016), the submission from this study is also in agreement with earlier findings (Scartezzini and Speroni 2000; Panda and Kar 2007), where plant-derived antioxidants proved effective in treating

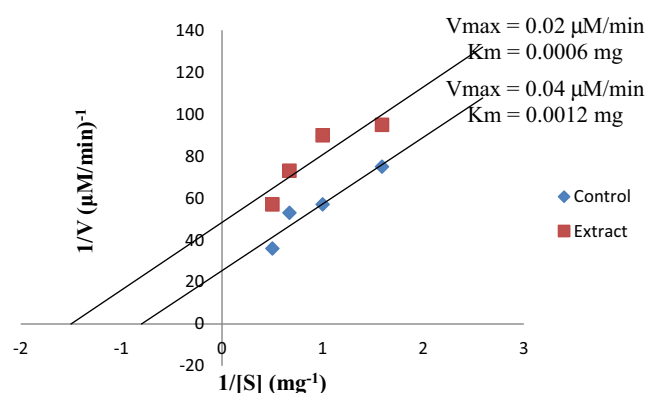


Fig. 2 The uncompetitive inhibitory effect of *S. incanum* fruit aqueous extract on alpha-amylase activity. (n = 3, mean ± SEM)

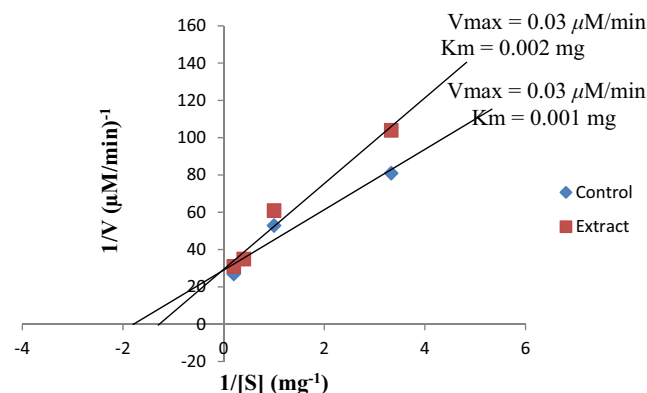
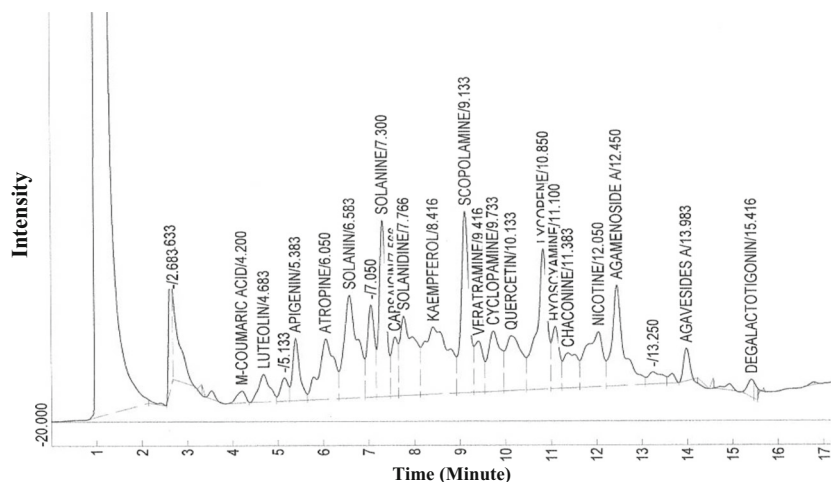


Fig. 3 Competitive inhibitory effect of *S. incanum* fruit aqueous extract on alpha-amylase activity. (n = 3, mean ± SEM)

Fig. 4 Gas chromatography-mass spectrophotometry chromatogram of *S. incanum* fruit aqueous extract



DM in vivo. Furthermore, plants endowed with significant antioxidant and anti-inflammatory potentials have also been shown to annihilate deleterious effect of lysosomal enzymes and facilitate membrane stabilization (Amujoyegbe et al. 2012). Judging by the results of the membrane stabilization potential of SIAE, it may be logically inferred that, SIAE at 2.0 mg/mL conferred marked stabilization potential (86.67%) on the RBC against hemolysis. Such effect might either be

adduced to the influence of SIAE to adhere to the erythrocyte membrane to prevent deleterious attacks on the membrane and promote dispersion of ions as normally observed in erythrocyte hemolysis. This potential of SIAE may be considered significant and supportive of its antioxidant effect that may provide further protection against free radicals-mediated β -cell injury, which by effect aided its regulatory capability on both glucose uptake by yeast cells and the specific activities of alpha-glucosidase and alpha-amylase.

Table 4 Adaptogenic constituents of aqueous fruit extract of *S. incanum*

Peak	RT	% PA	Component	MW (g/mol)	Class
1	4.20	0.68	M-coumaric	164.16	Cinnamic acid
2	4.6	1.96	Luteolin	286.24	Flavonoid
3	5.38	2.87	Apigenin	270.24	Flavone
4	6.05	5.68	Atropine	289.37	Alkaloid
5	6.58	8.92	Solanin	868.06	Saponin
6	7.30	7.55	Solanine	868.06	Saponin
7	7.57	2.34	Capsaicin	305.41	Capsiacinoid
8	7.77	7.20	Solanidine	397.64	Saponin
9	8.42	10.41	Kaempferol	286.23	Flavonoid
10	9.13	8.83	Scopolamine	303.35	Alkaloid
11	9.42	2.77	Vetramine	409.60	Alkaloid
12	9.73	4.73	Cyclopamine	411.62	Alkaloid
13	10.13	5.55	Quercetin	302.24	Flavonoid
14	10.85	9.34	Lycopene	536.87	Carotenoid
15	11.10	2.99	Hyoscyamine	289.38	Alkaloid
16	11.38	3.01	Chaconine	706.93	Alkaloid
17	12.05	5.84	Nicotine	162.23	Alkaloid
18	12.45	7.48	Agamenoside A	1211.20	Saponin
19	13.98	1.23	Agavesides A	1299.40	Glycoside
20	15.42	0.62	Degalactotigonin	1035.17	Glycoside

RT retention time (min), PA peak area, MW molecular weight

Plants with adaptogenic effect are rich source of complex phenolics or tetracyclic triterpenoids/steroids, which have multiple biological effects, including antioxidant and hypoglycemic activities (Abdelmoaty et al. 2010). Hence, the overall effects elicited by SIAE in the present report could be attributed to its phytonutrients with established bioactivities as revealed by the GC-MS data. Besides the antioxidant attributes of these phytoconstituents, the significant hypoglycemic effects of quercetin, luteolin, kaempferol, apigenin, cinnamic acid and capsaicin to improve insulin sensitivity, inhibit alpha-amylase and alpha-glucosidase activity, and regenerate pancreatic β -cells have been reported (Panda and Kar 2007; Abdelmoaty et al. 2010; Guo et al. 2015; Zhang et al. 2017). The marked influence of lycopene in ameliorating inflammatory response and oxidative stress linked to antidiabetic nephropathy has also been documented (Guo et al. 2015). Additionally, the identification and grouping of the other compounds as either alkaloids or saponins could further support the hypoglycemic potential of the extract. For instance, while alkaloids are known to enhance improved glycemic control (Shukla et al. 2012), the normalization of hemostatic imbalance and amelioration of oxidative stress in experimental animals have been associated with antidiabetic properties of saponins (El Barky and Hussein 2017). The overall synergistic effects of these adaptogenic phytonutrients in the fruit extract of SI might justify its medicinal property as a viable antidiabetic candidate.

Conclusion

The findings from this study have shown *S. inacanum* fruit aqueous extract as a potent antioxidant with significant membrane stabilization and hypoglycemic activities. This has not only lent credence to its folkloric usage but also suggested antioxidant, glucose uptake modulation, and carbohydrate-metabolizing enzymes' inhibition as its tentative mechanisms of hypoglycemic action.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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