



HPLC PROFILE, *IN-VITRO* ALPHA-AMYLASE, ALPHA-GLUCOSIDASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF *Gymnema sylvestre* ETHYL ACETATE LEAF EXTRACT

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ABSTRACT

Gymnema sylvestre (GS) has been used locally as antidiabetic, antioxidant, antihyperlipidemic and anticancer medicinal plant. Oxidative stress has been implicated in pathogenesis of various diseases including diabetes and antioxidants play a leading role in preventing the progression of the disease. This study aimed to determine antidiabetic potential relative to carbohydrate hydrolyzing enzymes (alpha-amylase and alpha-glucosidase) and antioxidant activities towards oxidative stress and lipid peroxidation (MDA) of *Gymnema sylvestre* ethyl acetate leaves extract (GSEALE). The extract was found to inhibit α -glucosidase than α -amylase with Inhibitory concentration (IC_{50}) values of 170.45 ± 13.61 and 130.77 ± 3.15 respectively. The extract was also found to scavenge DPPH ($53.87 \pm 17.12\%$), hydroxyl ($28.10 \pm 14.35\%$) and hydrogen peroxide ($34.26 \pm 20.12\%$) radicals. The activities of superoxide dismutase (SOD) was found to be significantly lower ($p < 0.05$) when the extract-treated and extract+ H_2O_2 -treated were compared with the normal control (untreated group). Levels of reduced glutathione (GSH) in the extract-treated was found to be significantly lower ($p < 0.05$) when compared with both normal and positive controls while the extract+ H_2O_2 -treated was significantly lower than the positive control but higher than extract-treated group. Levels of MDA was significantly higher in extract-treated and extract+ H_2O_2 -treated than the positive control ($p < 0.05$). The total flavonoids (9.29 ± 0.76) and total phenolics (6.18 ± 0.52) and saponins (62.98 ± 0.31) were found to be present in the leaves in high amount with tannins, steroids, soluble starch and carbohydrates also present. High Performance Liquid Chromatography profile revealed Resorcinol/Catechol to be one of the phenolics detected and may be responsible for GSEALE's inhibition of α -amylase and α -glucosidase and antioxidant properties. This shows that GS can probably be used for antidiabetic purpose due to significant antioxidant and carbohydrate hydrolyzing enzymes inhibitory properties.

Keywords: Antioxidants, HPLC, *Gymnema sylvestre*, carbohydrate hydrolyzing enzymes, lipid peroxidation, Diabetes mellitus

INTRODUCTION

Diabetes mellitus, (DM), is a chronic hyperglycaemia that may be caused by one or more of numerous underlying processes (WHO, 2014). Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications (WHO, 2014). Acute complications include diabetic ketoacidosis and non-ketotic hyperosmolar coma (Kitabchi *et al.*, 2009). Long-term complications of diabetes include cardiovascular diseases, stroke, chronic kidney failure, foot ulcers, and damage to the eyes (WHO, 2014). The disease is due to either the pancreatic beta cells not producing enough insulin or the cells of the body generally not responding properly to the insulin produced (Shoback, 2011). Millions of people in developing nations, including Nigerians, have resorted to the use of plants to treat their ailments; this could be due to the high cost of orthodox health care or as a result of the global shift towards the use of natural sources, rather than synthetic drugs (Omonkhua and Onoagbe, 2011).

Protein glycation and glucose autooxidation generates free radicals in diabetic patients, which in turn catalyses lipid peroxidation (Onorato *et al.*, 2000). The antioxidant status of the diabetic is compromised

and is unable to protect against free radical damage (Bonnefont-Rousselot *et al.*, 2000). It was documented that diabetes is associated with increased oxidative stress as evidenced by the increased accumulation of lipid peroxides (Maritim *et al.*, 2003). Under physiological conditions, antioxidant defense system protects the body against adverse effects of free radical generation. In diabetes mellitus hyperglycemia may depress the natural antioxidant system. Elevated generation of free radicals resulting in the depletion of antioxidant defense components may lead to disruption of cellular functions and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation. A study indicated that modified oxidative stress is due to chronic hyperglycemia (Bhor *et al.*, 2004). This free radical and related reactive species may cause oxidative stress, which produces major interconnected changes of cellular metabolism, increases the serum marker enzymes, DNA fragmentation, and destruction of the cells by lipid peroxidation (Bhadauria *et al.*, 2008). The lipid peroxides accumulated introduce hydrophilic moieties and thus alters membrane permeability balance and cell function which causes the loss of liver integrity and hepatic dysfunction resulting in hepatotoxicity.

One of the important methods adopted to treat diabetes is inhibition of carbohydrate-digesting enzymes such α -amylase and α -glucosidase in the gastrointestinal tract, with retardation of intestinal glucose absorption and lowering of postprandial blood glucose levels (Rhabasa-Lhoret and Chiasson, 2004). Alpha glucosidase cleaves glycosidic bonds in complex carbohydrate to release absorbable monosaccharides. Inhibitors of α -glucosidase display useful anti-hyperglycaemic effects (Stuart *et al.*, 2004). Acarbose and miglitol (standard drugs) are examples of competitive inhibitors of α -glucosidase (Davis and Granner, 1996).

Phenolics are the most widespread secondary metabolites in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant. The antioxidant activity of the plant extract is mainly due to presence of phenolic compounds due to their redox properties, hydrogen donors and singlet oxygen quenchers (Hatano *et al.*, 1989). The interests in phenolic compounds are increasing in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food (Aneta *et al.*, 2007). This study aimed to determine antidiabetic potential relative to carbohydrate hydrolyzing enzymes (α -amylase and α -glucosidase) and antioxidant activities towards oxidative stress and lipid peroxidation (MDA) of *Gymnema sylvestre* ethyl acetate leaves extract (GSEALE).

MATERIALS AND METHODS

Plant collection and Identification

The leaves of *Gymnema Sylvestre* were collected from *Filin Shagari*, Bauchi State, Nigeria. It was identified at the herbarium unit of Biological sciences department, Bayero University Kano and voucher specimen no. BUKHAN0349 was deposited for future references. The leaves were shade dried and ground into smooth powder using a clean metal mortar and pestle. It was weighed using a weighing balance and kept in clean polythene nylon. The sample was used throughout the experiment.

Preparation of Extract

Gymnema Sylvestre leaves powder (40g) was dissolved in 200ml of ethyl acetate. The container was quickly and well covered using an aluminum foil paper first followed by the container cover. After 48 hours, the mixture was filtered using a nylon sieve into the small container and the residue spread on a wide plastic plate and allowed to dry. The residue was re-extracted twice with fresh 200ml of ethyl acetate for 24 hours. The pooled and dried extract was used for this study.

Animal Acquisition and Care

Three (3) healthy rabbits and fifteen (15) mice were procured from *Sabon gari* Market, Kano, and animal house, Bayero University, Kano (BUK)-Nigeria respectively. They were kept in metal cages at the animal house of the department of Biological Sciences, BUK. They were allowed to acclimatize for one week and given access to food and water *ad libitum* following the method of Klein and Bayne (2007). The protocols of the study was according to international Test guidelines (TG407) (OECD, 2006) and also the

National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996).

Animal (mice) Grouping

The mice were grouped (n=3) into the following:

Group 1: Served as test group and received alloxan (150mg/kg) + 600mg/kg GSEALE.

Group 2: Served as the normal control and received 10ml/kg b.w water.

Group 3: Served as Diabetic control and received 150mg /kg body alloxan only.

Group 4: Served as standard drug group and received 150mg/kg alloxan + 6 mg /kg body weight of Glibenclamide

Induction of Diabetic model

Mice were made diabetic by a single intraperitoneal (i.p.) injection of alloxan monohydrate, dissolved in normal saline at a dose of 150 mg/kg b.w. (Yanarday and Colae, 1998).

Percentage change in Fasting Blood Glucose (FBS) was calculated by simple formula

$\% \Delta \text{FBS} = \frac{\text{Final (FBS at time } t = 2 \text{ or } 4\text{hrs)} - \text{Initial (FBS at } t=0)}{\text{Initial (FBS at } t=0)} \times 100$

Preparation of Rabbit Liver Slices

The rabbit liver was obtained fresh after the animal decapitation, plunged into cold sterile phosphate buffer saline, thin slices of 1mm was cut using a sterile scalpel and maintained freeze till use.

Phytochemical Screening

Chemical test to screen the phytochemicals was conducted on the crude powder of the plant sample using standard methods described by Sofowora (1993), Trease and Evans (2002), Vishnoi (1979), each of the tests was qualitatively expressed as negative (-) or positive (+).

Qualitative Phytochemical Analyses

Molisch's Test of Carbohydrates, Monosaccharides, Free reducing sugars, combined reducing sugars, Anthraquinones, Steroids, Terpenoids and saponins were carried out as outlined by Sofowora (1993) Method. Test for Tannins and Flavonoids was by Trease and Evans (2002). Soluble Starch was tested by Vishnoi (1979) Method.

Quantitative Phytochemical Analyses

Determination of Saponins and Alkaloids by Sofowora (1993); Flavonoids and Tannins/Pseudotannins by Trease and Evans (2002); Total Phenolic (Pyrocatechol Gallic Acid) Compounds (Mallick and Singh, 1980) and Total Flavonoid by Cameron *et al.* (1943).

High Performance Liquid Chromatography (HPLC) of *Gymnema sylvestre* Ethyl Acetate Leaves Extract (Gupta *et al.*, 2012)

The following methodology was used for obtaining the chromatogram of extract and five different standards; resorcinol, gallic acid, catechol, quercetin and saponin white. High Performance Liquid Chromatography (HPLC) analysis was performed using a Shimadzu LC20A System with Shin-pack VP-OSD (150mm×4.6mm i.d 5 μ m column) and LC-solution software. Five (5) mg of each standard compound and 0.5mg of the extract were dissolved in 10 ml of HPLC grade methanol resulting in a sample concentration of 500 μ g/ml.

This was sonicated and then passed through Whatman Nylon Membrane Filter (0.45µm and 47mm diameter) before injecting it in the column. Gradient elution of two solvents was used- Solvent A (Acetonitrile) and Solvent B (Methanol (1:25) (Gupta *et al.*, 2012). The gradient program was begun with 100 % B and was held at this concentration for the first 4 minutes. This was followed by 50 % eluent A for the next 6 minutes after which concentration of A was increased to 80 % for the next 10 minutes and then reduced to 50 % again for the following 2 minutes.

Evaluation of Radical Scavenging Effects of *Gymnema sylvestre*

The scavenging effects of *Gymnema sylvestre* were evaluated against DPPH, hydrogen peroxide and hydroxyl radicals.

DPPH Spectrophotometric Assay

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the method of Mensor *et al.* (2001).

Hydrogen Peroxide Scavenging Effects

The ability of the extracts to scavenge hydrogen peroxide was assessed by the method of Ruch *et al.* (1989).

Measurement of Hydroxyl Radical Scavenging Activity

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2- deoxyribose oxidative degradation as described by Elizabeth and Rao (1990) with modification where phosphate buffer was used instead of KH₂PO₄ – KOH buffer.

Antioxidant Activity (Non-Enzymatic Antioxidants)

Estimation of Reduced Glutathione

Reduced glutathione was determined by the method of Moron *et al.* (1979).

Enzymatic Antioxidants

The enzymatic antioxidants analyzed include superoxide dismutase and catalase. All the assays were carried out in triplicate.

Assay of Catalase (CAT, EC 1.11.1.6)

Catalase activity was assayed using the method of Luck (1974).

Assay of Superoxide Dismutase (SOD, EC 1.15.1.11)

SOD was assayed according to the method of Kakkar *et al.* (1984) with modification. Phosphate buffer (0.1 M; pH 7.4) was used instead of 0.025M sodium pyrophosphate buffer (pH 8.3) and potassium phosphate buffer (50mM, pH 6.4).

Estimation of Lipid Peroxidation (LPO) In Rat Liver Slices

The extent of LPO in rabbit liver slices was estimated by the method described by Nichans and Samuelson (1968).

In-vitro α-Amylase (EC3.2.1.1) and α-Glucosidase ((EC3.2.1.20) Inhibition Assay

The α-amylase and α-glucosidase inhibitory activities were determined according to the method described by Kim *et al.* (2000) and Jung *et al.* (2006).

Statistical Analysis

Data are presented as means ± SEM. Comparison between the means was done using student t-test, p<0.05 considered significant using SPSS Statistics 17.0 version. Percentage change of glucose level was calculated using Microsoft Excel.

RESULTS

The results obtained for the phytochemical screening and the radical scavenging activities, in-vitro antioxidant activities, lipid peroxidation, inhibition effects of carbohydrate hydrolysing enzymes (α-amylase and α-glucosidase) and hypoglycaemic effects of *Gymnema Sylvestre* ethyl acetate leaves extract are presented in the tables below.

Table 1: Phytochemical Composition of *Gymnema sylvestre* Ethyl acetate leaf Extract.

PHYTOCHEMICALS	QUALITATIVE
Saponins	+
Flavonoids	+
Alkaloids	+
Tannins	+
Carbohydrates	+
Monosaccharides	-
Free reducing sugars	+
Combined reducing sugars	-
Anthraquinones	-
Steroids	+
Soluble starch	+
Terpenoids	+

+ = present; - = absent; NA = Not assayed.

Table 2. Quantitative Phytochemical Composition of *Gymnema sylvestre* Ethyl acetate leaf Extract.

PHYTOCHEMICALS	QUANTITATIVE (%)
Saponins	62.07±0.31
Flavonoids	0.66±0.02
Alkaloids	13.90±0.15
Tannins	10.09±0.26

Table 3: HPLC Profile of Ethyl Acetate Extract of *G. sylvestre* compared with some phenolic Standards

S/N	RT	Compound	Molecular Weight	Molecular Formular	Area (%)
1	3.259	Catechol	110.10	C ₆ H ₆ O ₂	100.00
2	4.402	Gallic acid	170.12	C ₇ H ₆ O ₅	100.00
3	3.366	<i>G. sylvestre</i>	-	-	68.152
4	12.096	Quercetin	302.24	C ₁₅ H ₁₄ O ₉	96.574
5	3.421	Resorcinol	110.10	C ₆ H ₆ O ₂	100.00
6	2.862	Saponin white	414.00	C ₂₇ H ₄₂ O ₃	53.369

RT= Retention Time

Table 4: Radical scavenging activity of *Gymnema sylvestre* Ethyl Acetate Leaf Extract.

RADICAL SCAVENGER	%COMPOSITION
Total phenols (µg/ml)	6.18±0.52
Total flavonoids (ng/ml)	9.29±0.76
DPPH scavenging activity (%)	53.87±17.12
H ₂ O ₂ scavenging activity (%)	34.26±20.12
OH radical scavenging activity (%)	28.10±14.35

Values are represented as mean ± standard error of mean (n = 3).

Table 5: *In-vitro* antioxidant activity and lipid peroxidation of *Gymnema sylvestre* ethyl acetate leaves extract in rabbit liver slices.

GROUPS	Enzymatic Antioxidants				Lipid peroxidation (MDA) (10 ⁻¹ (mmole/TBARS)
	Superoxide dismutase (SOD)(U/ml)	Catalase (CAT) (U/ml)	Reduced Glutathione (GSH) (mg/ml)		
Untreated (control)	2.20±0.02	275.23±231.00	3.60±0.18		17.05±4.94
H ₂ O ₂	2.07±0.02 ^a	630.73±914.85	5.35±0.57 ^a		11.86±1.96
GSEAL Extract	2.07±0.02 ^a	11.47±56.47	1.74±0.14 ^{a,b}		30.28±6.32 ^b
H ₂ O ₂ +extract	2.09±0.01 ^a	286.70±129.24	3.57±0.33 ^{b,c}		19.91±1.08 ^b

Values are mean ± standard deviations; n=3; with those bearing different superscripts a, b and c under the same column being respectively significant with untreated (control), H₂O₂ and Extract induced using student t-test (p<0.05).**Table 6: Percentage Inhibition of α-Amylase By *Gymnema sylvestre* Ethyl Acetate Leaves Extract (GSEALE)**

Conc (mg/ml)	Alpha-amylase				IC ₅₀ (mg/ml)
	% inhibition				
Standard acarbose	4	8	15	20	200.19±7.29
GSEALE	9.10±1.10	29.35±0.35	58.05±0.05	75.15±2.95	170.45±13.61
	15.40±1.40 ^a	31.50±0.50 ^a	43.35±0.25 ^a	74.30±3.10	

Values are mean ± standard deviations; n=3; with those bearing superscript a under the same column being respectively significant with standard acarbose using student t-test (p<0.05).

Table 7: Percentage Inhibition of α-Glucosidase By *Gymnema sylvestre* Ethyl Acetate Leaves Extract (GSEALE)

Conc (mg/ml)	Alpha- glucosidase				IC ₅₀ (mg/ml)
	% inhibition				
Standard acarbose	4	8	15	20	182.26±1.05
GSEALE	31.00±0.30	46.15±3.15	58.70±1.30	88.10±0.20	130.77±3.15 ^a
	30.5±0.20	39.65±1.35	60.80±0.20	62.60±1.60 ^a	

Values are mean ± standard deviations; n=3; with those bearing superscript a under the same column being respectively significant with standard acarbose using student t-test at p<0.05.

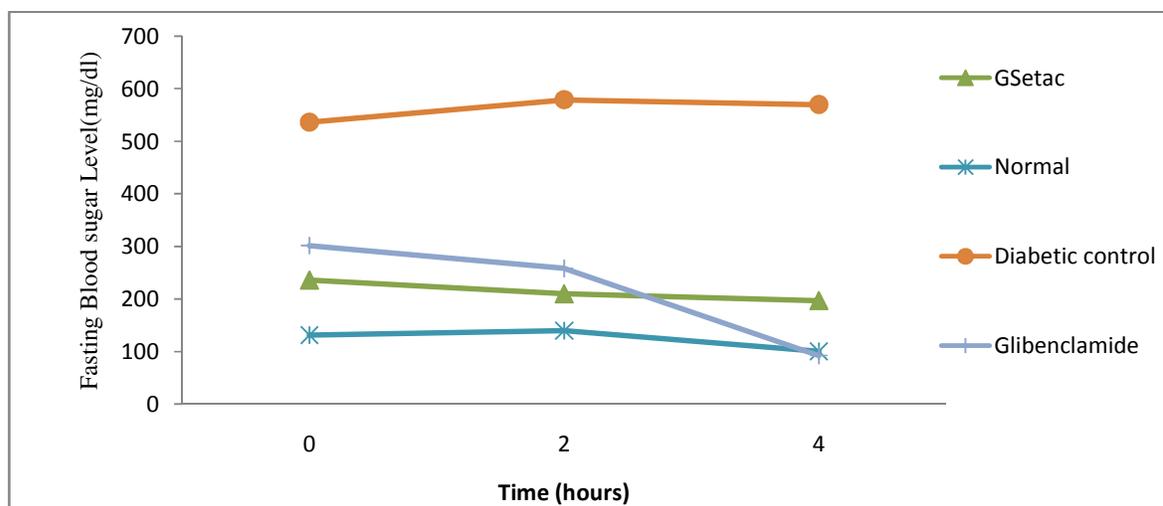


Fig. 1: Mean Fasting Blood sugar Level (mg/dl) of Alloxan-induced Diabetic Mice Administered *G. sylvestre* ethyl acetate leaf extracts

Table 8: Percentage (%) Change in Fasting Blood Sugar of Alloxan-induced Diabetic mice Administered *G. sylvestre* Ethyl acetate Leaf Extract for Four Hours

Extract Type	% Change in Fasting Blood Sugar	
	2hr	4hr
Group 1 (GSetac)	-11.02 ± 0.80 ^{a,b}	-16.53 ± 2.10
Group 2 Normal	+6.62 ± 5.06 ^a	-23.41 ± 6.67 ^a
Group3(Diabetic control)	+7.92 ± 0.99 ^b	+6.24 ± 16.68
Group4 (Glibenclamide)	-14.43 ± 6.65	-69.32 ± 9.25 ^a

Values expressed as Mean ± SEM with those bearing same superscript under the same column significantly different (P < 0.05) with respective controls; N = 3. (+) preceding a value means % increase in Fasting Blood Sugar; (-) preceding a value means % decrease in Fasting Blood Sugar; GSEtac= *G. sylvestre* ethyl acetate extract.

DISCUSSION

Phytochemical analysis conducted on *Gymnema sylvestre* ethyl leaves extract (GSEALE) revealed the presence of tannins, flavonoids, steroids, saponins, terpenoids, alkaloids, soluble starch, carbohydrate and free reducing sugars (Table 1). Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Aiyegoro and Okoh, 2010). It is also reported by Eleazu and Okafor (2012) that tannins inhibit the activities of digestive enzymes such as trypsin, chymotrypsin, amylase and lipase. The tannin-epigallo-catechin-3-gallate is known to possess hypoglycemic activity.

Flavonoids function as health promoting compounds due to its anion radicals scavenging properties (Aiyegoro and Okoh, 2010). In addition, flavonoids, as antioxidants may prevent the progressive impairment of pancreatic beta cell function due to oxidative stress, thereby reducing the occurrence of diabetes (Eleazu and Okafor, 2012). These observations support the usefulness of this plant (GS) in traditional remedies in the treatment of stress-related ailments such as hypertension and diabetes (Ferguson, 2001). Also, the plant extract was revealed to contain saponins, known to produce inhibitory effect on inflammation (Aiyegoro and Okoh, 2010), and are major ingredients in traditional medicine and thus

responsible for most of the observed biological effects (Liu and Henkel, 2002), and this justify the use of *Gymnema sylvestre* in traditional medicine as anti-inflammatory plant.

The presence of these phenolic compounds in this plant might contribute to their antioxidative properties and thus the importance of this plant in traditional herbal medication. The plant is used routinely among many tribes in Africa for the treatment of various diseases such as diabetes. Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitors of key enzymes like alpha amylase and alpha glucosidase associated with type 2 diabetes and lipid peroxidation in tissues (Reddy *et al.*, 2010).

According to Valko *et al.* (2007) antioxidant treatment suppresses apoptosis in β-cells without changing the rate of β-cell proliferation, thus, supporting the hypothesis that says in chronic hyperglycemia, apoptosis induced by oxidative stress causes reduction of β-cell mass and hence the level of insulin. The plant extract was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone (Okwu, 2001). This is further corroborated by the studies of Daisy *et al.* (2009) and El-Shafey *et al.* (2013), which found a novel steroid with antidiabetic activity,

thus, suggesting steroids to be involved in stimulating pancreatic β - cells and subsequent secretion of insulin, hence useful in treating diabetes.

High performance liquid chromatography (HPLC) was done on GSEALE. The chromatogram of the extract was compared with that of standard catechol, gallic acid, quercetin, saponin white and resorcinol. Chromatograms peaks, retention times and molecular features comparisons revealed the polyphenol in GSEALE to be either resorcinol or catechol (Table 3). Thus, this indicated resorcinol and catechol present in GSEALE might be responsible for the biological activities of the extract observed in this study.

The result of DPPH scavenging activity (Table 4) assay in this study indicates that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Hydrogen peroxide is an important reactive oxygen species (ROS) because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell (Gulcin *et al.*, 2003). Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics content (table 1 and 3), which can donate electron to H_2O_2 , thus reducing it to water. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism (Parr and Bolwell, 2000).

The enzyme system SOD-CAT represents the first line of defence against free radicals molecular atrocities. SOD catalyses the dismutation of the superoxide anion radical. As a result, H_2O_2 is produced and decomposed by the CAT. The study shows decreased SOD and CAT activities in the H_2O_2 -induced GS leaves when compared with untreated controls and this slight depletion in the levels of the enzymes could be attributed in their involvement in the scavenging of H_2O_2 . In the case of extract-treated liver slices, significant decreases of SOD and GSH activities were also observed which could be attributed to the same reason as earlier stated (Table 5). The capability of the GSEALE to ameliorate these decreases could offer protective importance and thus protect against tissue damage and cell death. These findings also agreed with the work of Lukaszewicz-Hussain and Moniuszko-Jakoniuk (2003).

The concentration of GSH, a key cellular non-enzymatic antioxidant, was also determined (Table 5). Numerous enzymes participate in glutathione metabolism. Depleted glutathione levels after the various treatments may be caused by its involvement in scavenging of H_2O_2 (Lukaszewicz- Hussain and Moniuszko- Jakoniuk, 2003). Especially, this is *in vitro* and the GSH-GSSG cycle might not work in dead cells. Oxidative stress affects cellular integrity only when anti-oxidants are no longer capable of coping with ROS. ROS reacts with the unsaturated fatty acid of cellular or subcellular membranes. Therefore, they lead to peroxidation of membrane lipids. The oxidative stress caused by different xenobiotics is often estimated by the level of MDA. In this study, there is a decrease level in MDA of H_2O_2 induced liver slices

while an increase level in MDA in other treatments. The observed increase in MDA in this study may be associated with glucose autooxidation. Since hyperglycemia-induced oxidative stress occurs in non-nucleated cells lacking mitochondria and the NAD(P)H oxidase (erythrocytes), another mechanism of ROS formation in such cells must exist. A possible explanation for such behaviour is glucose auto-oxidation (Robertson *et al.*, 2003). Glucose itself, as well as its metabolites, is known to react with hydrogen peroxide in the presence of iron and copper ions to form hydroxyl radical and subsequent generation of MDA as observed in this study.

The present study indicated that the plant extracts produced a better alpha-glucosidase enzyme inhibition (IC_{50} 130.77 \pm 3.15) when compared with alpha-amylase (IC_{50} 170.45 \pm 13.61) and also when both are compared with standard acarbose (IC_{50} 182.26 \pm 1.05 and 200.19 \pm 7.29) respectively (Table 6). Thus, *Gymnema sylvestre* could be useful in management of postprandial hyperglycemia than the presently used synthetic enzyme inhibitors (e.g acarbose) which cause gastrointestinal side effects such as diarrhea, flatulence, abdominal bloating e.t.c (Kavitha *et al.*, 2012). The inhibitory effect of this plant may be attributed active principles such as polyphenols, flavonoids and glycosides as reported by Eleazu and Okafor (2012) and Jung *et al.* (2006). This was further supported by *in vivo* study that demonstrated significant change in blood glucose in alloxan-induced experimental mice when administered with extract after two (2) and four (4) hours (Table 8).

Alpha-amylase and glucosidase inhibitors are the potential therapeutic targets in the development of lead compounds for the treatment of diabetes (Subramanian *et al.*, 2008). In animals system, alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha-amylase can convert starch to simple sugars thus controlling blood glucose levels (Mohammed *et al.*, 2009; Kumanan *et al.*, 2010). This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood (Mohammed *et al.*, 2009). Hence diabetics tend to have low alpha-amylase levels in order to keep their glucose levels under control. This may justify the presence of inhibitors of pancreatic α -amylase in GSEALE which can delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the postprandial serum glucose levels (Table 8).

Inhibition of these enzyme systems helps to reduce the rate of digestion of carbohydrates (Bhat *et al.*, 2011). Therefore, natural alpha-amylase and glucosidase inhibitors from the dietary/medicinal plants can be used as an effective therapy for treating post prandial hyperglycemia with minimal side effects as revealed by this study.

CONCLUSION

In this study, GSEALE showed a remarkable *in vitro* antioxidant, α - glucosidase and α -amylase inhibitory potentials. The inhibitory effect of key enzymes relevant to type 2 diabetes was found to be better than that of the standard drug, acarbose.

This could be attributed to the presence of phytochemicals especially resorcinol, catechol and other polyphenolic compounds, present in the extract as confirmed by HPLC profile, hence the plants can be used as an alternative therapy for treating post prandial hyperglycemia.

Conflict of interest

Authors declared that no conflict of interest.

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