IN-VITRO ANTIOXIDANT, ANTIGLYCATION AND ANTI-LIPID PEROXIDATION ACTIVITIES OF *Sorghum bicolor* METHANOLIC LEAF SHEATH EXTRACT

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Abstract: This study was conducted to determine the in-vitro antioxidant, anti-lipid peroxidation and antiglycation activities of *Sorghumbicolor* methanolic leaf sheath extract using standard procedures. Concentration of antioxidant compounds, β-carotene, lycopene, total flavonoids and total phenolics in the plant extract are 71.74 and 88.84 µg/100mL, 11.68 ± 0.05 and 20.45 ± 0.12 mg/g, respectively. *Sorghum bicolor* methanolic leaf sheath extract showed a considerable high antioxidant activity when compared to ascorbic acid by scavenging DPPH and showing reducing property in a dose dependent manner. It also possessed the ability to inhibit lipid peroxidation and proteinglycation. From the result obtained, *Sorghum bicolor* methanolic leaf sheath extract contains bioactive components which possess antioxidant, antiglycation and anti-lipid peroxidation activity, thus, can be utilized to delay the onset of diabetes and diabetic complications.

Keywords: Antioxidant, antiglycation, anti-lipid peroxidation and *Sorghum bicolor* leaf sheaths

Introduction

Diabetes is a common metabolic disease responsible for 2% of deaths arising from non-communicable diseases in Nigeria (WHO, 2014). It is a disorder characterized by blood hyperglycemia due to inability of the pancreas to secrete insulin or insensitivity of the insulin secreted (Akoro *et al*., 2017). Oxidative stress has been implicated in the etiology of this disease. Oxidative stress is a condition arising from overproduction of free radicals *in-vivo* as against the body's antioxidant system equipped to neutralize, scavenge and quench these radicals. Glycation and lipid peroxidation are vital biological processes that aids the incidence of diabetes and its complications like neuropathy, retinopathy, nephropathy, and cardiovascular disease alongside oxidative stress (Sharifi-Rad *et al*., 2017; Sugimoto *et al*., 2008; Fukami *et al*., 2008; Yamagishi *et al*., 2008). These two processes have one thing in common, that is, disruption of cellular integrity and loss of biological function, thus compromising the health of individuals (Gutierrez *et al*., 2012).

Many researchers across the world now focus on medicinal plants that can prevent the onset of diabetes and as well manage its complications. Plants' natural products make excellent lead for the development of new drugs used for the prevention, management and treatment of various communicable and non-communicable diseases (Omotayo *et al*., 2017). Since time immemorial, public health has focused more on the prevention of communicable diseases; however, the emergence of the 20th century caused a foci shift to non-communicable diseases including diabetes, cancers and cardiovascular diseases arising from sedentary life style, diet, pollution and use of advanced electronic gadgets (Ogundare *et al*., 2020). Available are orthodox drugs and sophisticated equipment for treatment of these diseases which are quite expensive accompanied with numerous side effects. Thus, large populations in Nigeria now rely on medicinal plants or herbal medicine for therapeutic succor.

Sorghum bicolor is a common plant crop with many nutritional and medicinal benefits commonly referred to as "poroporooka baba". The leaf is a famous medicinal plant part commonly used as a recipe in the treatment of anaemia by traditional health care practitioners (Erah *et al*., 2003; Oladiji *et al*., 2007) and this claim has been substantiated by various researchers in Nigeria. Ogwumike (2002) reported the generation and hemoglobin-content of erythrocytes in experimental rats, while Akande *et al*. (2010) validates the hepatoprotective and hematopoietic effects of sorghum leaf sheaths and the central antinociceptive properties of sorghum was given by Nwinyi *et al*. (2009). This study evaluates the in-vitro antioxidant, anti-lipid peroxidation and antiglycation activity of *Sorghum bicolor* methanolic leaf sheath extract.

Materials and Methods

Collection and identification of plant

Sorghum bicolor leaf sheath was purchased from a local herbal market (Mushin) in Lagos, Nigeria. The test plant was identified by Dr. Nodza George and deposited in the University of Lagos Herbarium with number LUH 8548.

Extraction of plants materials

The *Sorghum bicolor* leaves were washed under running tap water, drained and dried under shade until constant plant weight was obtained. The plant was milled using electric blender to obtain coarse powder and kept in air tight container until required for extraction. Active constituents of SBLS (100 g) were extracted with methanol by maceration for 72 h. The filtrate was concentrated to dryness using a vacuum rotary evaporator to give a percentage yield of 12.84%.

Preliminary phytochemical screening

Phytochemical examinations were carried out for all the extracts using the standard methods as described by Sofowora (1993).

Determination of β-carotene and lycopene content of the extracts

Lycopene and β-carotene content of the extracts were evaluated using the method of Nagata and Yamashita (1992). The methanolic leaf extract of *S. bicolor* (0.1 g) were each weighed separately into a beaker, acetone:hexane mixture (4:6, 10 mL) was added and vigorously shaken for 5 min, filtered using Whatman paper (No. 42) and the absorbance of the filtrate was measured at 453, 505, and 663 nm. The concentration of β-Carotene and lycopene in the plants extracts were calculated using the following equations:

β-carotene (mg/100 mL) = 0.216 (A_{663}) - 0.304 (A_{505}) + 0.452 (A_{453})

Lycopene (mg/100 mL) = -0.0458 (A_{663}) + 0.372 (A_{505}) + 0.0806 (A_{453})

Total phenolic content

The total phenolic contents of both extracts were determined using the method of Chun *et al.* (2003). Folin-Ciocalteu's reagent (1 mL) was added to sample (1 mL, 1.0 mg/mL) and mixed thoroughly. To this mixture, 4 mL of sodium carbonate (7.5%) and 10 mL of distilled water were added and

thoroughly mixed. The mixture was allowed to stand for 90 min at room temperature and absorbance of the reaction mixture was taken at 550 nm. The total phenolic content was extrapolated using a calibration curve (R^2= 0.9699) for gallic acid. The results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate and values expressed as mean ± SD.

Total flavonoid assay

Total flavonoid content of both extracts was measured by aluminum chloride colorimetric assay described by Chang *et al*. (2002). Each plant extract (0.5 mL, 1.0 mg/mL) was separately mixed with methanol (1.5 mL), $AlCl_3$ (0.1 mL, 10%), sodium acetate (0.1 mL, 1M) and distilled water (2.8 mL). The reaction mixture was left for 30 min at room temperature and the absorbance was read at 415 nm. The total flavonoid content was calculated using a calibration curve (R^2= 0.9961) for quercitin. All samples were analyzed in triplicate and values expressed as mean ± SD.

DPPH free radical scavenging activity

The free-radical scavenging activity of both extracts was measured as decrease in the absorbance of methanol solution of DPPH. DPPH solution (0.033 g/L in methanol; 5 mL) was added to 1 mL of extract solution at different concentrations (25, 50, 75, 100 µg/mL). After 30 min, absorbance was measured at 517 nm and compared with standards ascorbic acid and gallic acid. The DPPH scavenging activity was expressed as the percentage inhibition extrapolated using the formula:

% scavenging activity =

$$\frac{control\ absorbance - sample\ absorbance}{control\ absorbance} x\ 100$$

Where: Control Absorbance is absorbance of stock DPPH

IC_{50} was deduced from calibration curve by plotting concentration of plant extract against their corresponding percentage scavenging activity.

Reducing power assay

The extract was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. 1.0 ml of trichloroacetic acid (10%) was added to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (1.5 mL) was mixed with distilled water (1.5 mL) and $FeCl_3$ (0.1 mL, 0.1%) after mixing, the contents were incubated for 10 min and the absorbance was measured at 700 nm (Yen and Chen, 1995).

In-vitro antilipid peroxidation assay

Freshly excised goat liver (2 g) was processed to obtain 10% homogenate in cold buffered saline, pH 7.4 and centrifuge in order to get a clear tissue homogenate. 500 µL of different concentrations of the extracts was added to 3 mL tissue homogenate. Then, the lipid peroxidation was initiated by adding 100 µL of 15mM ferrous sulphate solution. After 30 min, 100 µL of thin reactions observed in various test tubes the reaction mixture indicated increased reducing power. Ascorbic acid was used as a positive control (was taken in a tube containing 1.5 mL of 10% TCA. After 10 min, tubes were centrifuged and mixed to 1.5 mL of 0.67% TBA in 50% acetic acid. The resulting supernatant obtained after centrifuging was collected into a clean test tubes and were heated in a boiling water bath, cooled and intensity of the pink coloured complex formed was measured at 535 nm (Prasanth *et al*., 2000).

Determination of antiglycation activity

Bovine Serum Albumin (500 µL) was incubated with glucose (400 µL, 500mM) and 100 µL of extract, 100 µL phosphate buffer saline (7.4) was added, and the reaction were allowed to proceed for 24 hrs at 60°C. The reaction was terminated by adding 10 µL of 100% TCA (trichloroacetic acid) and kept at 4°C for 10 min. The mixture was centrifuged for 4 min at 13000 rpm and the precipitate obtained was re-dissolved in 2.5 mL alkaline phosphate buffer saline (pH 10). The resulting mixture obtained was quantified for the relative amount of glycated BSA using Spectrofluorimeter. The excitation and emission wavelength used were at 370 nm and 440 nm respectively. (Matsuura *et al*., 2002)

Statistical analysis

All data are expressed as the mean ± standard deviation from at least three independent experiments.

Results and Discussion

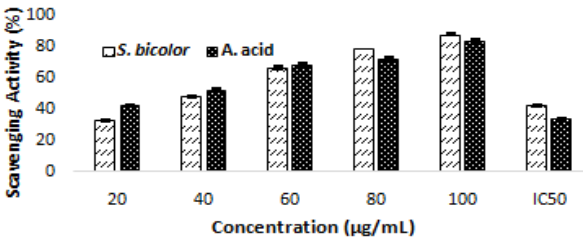
Polyphenolic compounds like flavonoids, phenolic acids and tannins are believed to be excellent natural antioxidants which are widely distributed and abundant in various families and species of plants (Choudhary *et al.*, 2013; Mazandarani *et al.*, 2012). These compounds play important roles in human health with beneficial effects like antiviral, anti-allergic, anti-thrombotic, anti-inflammatory, antioxidant and antitumor which aids the prevention of various human diseases (Konaté *et al.*, 2014; Kanti and Syed, 2009; Nair *et al.*, 2008; Kwon *et al.*, 2005). The antioxidant ability of flavonoids bestows a therapeutic potential in treatment and prevention of oxidative stressed related diseases including gastric or duodenal ulcers (Moreira *et al*., 2004), cardiovascular diseases and various cancers (Yang *et al.*, 2000; Yao *et al.*, 2004). In this study, the total phenolic and flavonoid content were estimated to be 11.68 ± 0.05 and 20.45 ± 0.12 mg/g of the plant sample, respectively.

Carotenoids are wide group of natural pigments responsible for the yellow, orange, and red colors of fruits, roots, flowers, fish, invertebrates, and birds. β-Carotene and lycopene are the major carotenoids (Alam and Sultan, 2004) which performs important role in protection against photooxidative processes by acting as oxygen and peroxylradical scavengers, quenching singlet oxygen and other electronically excited molecules thus, preventing the progression of many degenerative diseases in humans (Jacques *et al*., 2008; Lien *et al*., 2008). The lycophene and β-carotene concentration in *Sorghum bicolor* methanolic leaf extract were shown in Table 1 to be 88.84 µg/mL and 71.74 µg/100mL, respectively. Though, the concentration of lycopene and β-carotene in the plant extract is rather minimal, however their presence in the plant extract will add up to the antioxidant capability of the plant extract (Omotayo *et al*., 2020).

Table 1: Concentration of antioxidant compounds in *Sorghum bicolor* methanol leaf sheath extract

β-carotene (µg/100mL)	Lycophene (µg/100mL)	Total Phenolics (mg/g)	Total flavonoids (mg/g)
71.74 ± 0.21	88.84 ± 0.02	11.68± 0.05	20.45± 0.12

Values are represented as mean± SD of triple determinants



Value expressed as mean ± SD of three determinants

Fig. 1: DPPH radical scavenging activity of *Sorghum bicolor* methanol leaf sheath extract

The DPPH radical scavenging assay is a widely used method employed to assess the aptitude of plant extracts to scavenge free radicals attributed to their ability to donate hydrogen atoms (Philips *et al*., 2010; Chanda *et al.*, 2011; Eleyowo *et al*., 2018). Results shown in Fig. 1 indicate good DPPH scavenging activity indicated in the increase observed as the concentration of extract and ascorbic increased but considering their IC_{50} value, the *S. bicolor* methanolic leaf extract had greater activity (33.44± 0.12 mg/mL) compared to ascorbic acid (42.1±0.02 mg/mL).

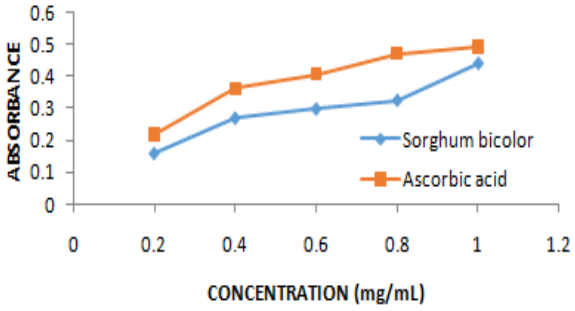


Fig. 2: Reducing potential of *Sorghum bicolor* methanol leaf sheath extract

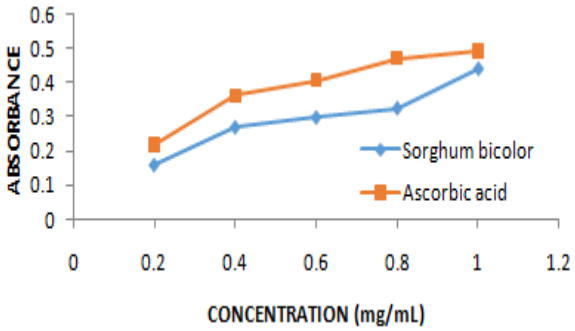
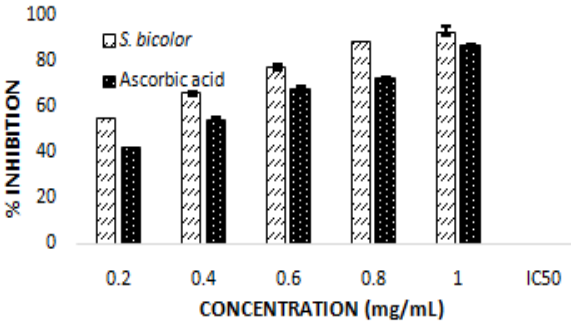


Fig. 2: Reducing potential of *Sorghum bicolor* methanol leaf sheath extract

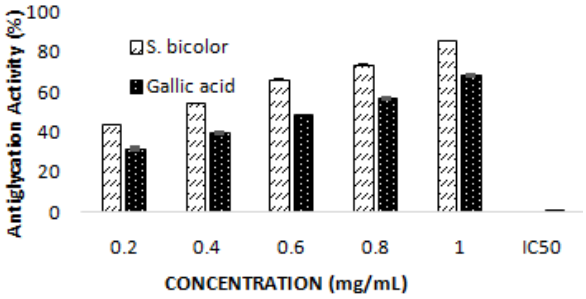
The reducing power assay is another important marker of potential source of antioxidant in plant extracts. This assay measures the intensity of the blue-green solution which absorbs at 700 nm resulting from reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}). It is related to the availability of atoms which can donate electron to free radicals and convert them to more stable metabolites and stop the radical chain reaction (Phaniendra *et al.*, 2015). The reducing power of the extract (Fig. 2) was found to increase with increasing concentrations compared to ascorbic acid indicating a reducing capacity. The result obtained in this study is comparable to that obtained by Omotayo *et al*., (2020) for methanolic extract of *Anacardium occidentale* and *Psidium guajava* methanolic leaf extracts.

Lipid peroxidation is a process induced by the effect of reactive oxygen species and reactive nitrogen species on fatty acids, lipoproteins, and phospholipids resulting to the formation of products like malondialdehyde, 4-hydroxynonenal and hydroperoxide lipid. These products damage the plasma membrane and may diffuse to other cells within the organism leading to inflammation through the binding of the oxidized low-density lipoprotein receptor and also triggering apoptosis (Lazo-de-la-Vega-Monroy and Fernndez-Mej, 2013; Liou and Storz, 2010). In this study, both SBLS extract and ascorbic acid inhibited lipid peroxidation in a dose dependent manner (Fig. 3). However, considering their IC_{50}, SBLS extract gave a stronger inhibition at a concentration of 0.08± 0.21 mg/mL while ascorbic had a value of 0.32± 0.02 mg/mL.



Value expressed as mean ± SD of three determinants

Fig. 3: Inhibition of lipid peroxidation by *Sorghum bicolor* methanol leaf sheath extract



Value expressed as mean ± SD of three determinants

Fig. 4: Antiglycation activity of *Sorghum bicolor* methanol leaf sheath extract

The antiglycation activity of SBSL methanol extract was shown in Fig. 4. The antiglycation activity of *Sorghum bicolor* metanolic leaf sheath extractgallic acid was dose dependent that is; increased percentage inhibitory activity was observed with increasing concentration producing IC_{50} of 0.32±0.01 and 0.62±0.01 mg/mL, respectively.

Conclusion

From the result obtained, *Sorghum bicolor* methanolic leaf sheath extract contains bioactive components which possess both antioxidant, antilipid peroxidation and antiglycation activity, thus, can be exploited for new drugs to delay the onset of diabetes and diabetic complications.

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