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Original Research Article

Antioxidants and Radical Scavenging Activities of Nigeria Soybeans (*Glycine max* (L.))

ABSTRACT

Aim: To evaluated the antioxidant and radical scavenging ability of three different accessions (TGx-1835-10E, TGx-1987-62F and TGx 1951-3F*)* of soybean.

Study Design: *In vitro* evaluation of antioxidant assays: Total phenol, Total flavonoid, Total antioxidant capacity, Ferric reducing antioxidant capacity, Cupric reducing antioxidant capacity, Ferrous Ion-chelating Ability, DPPH and NO radical scavenging activities.

Place and Duration: Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria
(August–December, 2016).

Methodology: Hydroalcoholic crude extracts of TGx-1835-10E, TGx-1987-62F and TGx 1951-3F were obtained through soxhlet apparatus using 80% methanol and concentrated in a rotary evaporator at 40^{O}C. The crude extract was then subjected to different complementary antiradical scavenging assays (Total phenol, Total flavonoid, Total antioxidant capacity, Ferric reducing antioxidant capacity, Cupric reducing antioxidant capacity, Ferrous Ion-chelating Ability , DPPH and NO radical scavenging activities.) following standard procedures.

Results: The results shows that TGx 1951-3F elicited the highest DPPH and NO radical scavenging activity with IC_{50} value of 2.61± 0.02 mg/ml and 2.58 ± 0.02 mg/ml, compared to TGx-1835-10E and TGx-1987-62F. Similarly, Ferrous Ion-chelating Ability (FIC) of TGx 1951-3F was higher with IC_{50} value of 1.38 ± 0.07 mg/ml, compared to TGx-1835-10E and TGx-1987-62F with an IC_{50} of 1.86 ± 0.16 and 2.07 ± 0.16 mg/ml. The reducing power of the three accessions expressed in terms of ascorbic acid equivalent tested using FRAP, TAC and CUPRAC assays showed that TGx 1951-3F has highest antioxidant activity follow by TGx-1835-10E and TGx-1987-62F. This same trend was also observed in antioxidant constituent present in the samples as TGx-1951-3F has higher phenolic and flavonoid content compared to TGx-1835-10E and TGx-1987-62F.

Conclusion: The result of this present study revealed that Accession TGx 1951-3F elicit the highest antioxidant potential nevertheless, accessions TGx-1835-10E and TGx-1987-62F also contain significant amounts of flavonoids and phenolic compounds. Consequently, making the plant seeds an important source of natural antioxidant, and might be helpful in prevention and management of various diseases associated with oxidative stress.

KEY WORDS: soybean, antioxidants, radical scavengers, oxidative stress

1. INTRODUCTION

Soybeans (*Glycine max* L.) a legume that is universally consumed. Soybeans is a complex food matrix containing no or low starch, 9% water, 30% carbohydrates, 20% total fat and 36% protein in addition to wide arrays of bioactive phytochemicals like isoflavones, lunasin, saponin, and trypsin inhibitors [1]. Recently there is a growing interest in Soybeans by researchers owing to its potential role in the prevention of a number of chronic degenerative diseases like cancer, coronary heart disease and osteoporosis [2]. Consumption of soybeans have become so widely important in recent times because of it human benefit such as protect heart health, defend against cancer, reduce the effects of menopause, improve digestive health, reduce risk of hypertension and decrease the risk

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of diabetes which was adduced to the present of phenolic, isoflavone [3-4]. Bioactive phytochemicals present in soybeans differ greatly with the cultivar, weather and geographical planting location [5-6]. It has been recorded that Indian cultivars are rich in genistein content compared to the European and American soybean cultivars. [7].

Reactive oxygen species (ROS) and free radicals are constantly produced in pathological conditions and has become a normal physiological bane [8]. Reactive species such as hydroxyl radical (OH˙), hydrogen peroxide, superoxide anions (O2-), and nitric oxide react with DNA, proteins, and lipids that eventually lead to cell death and tissue damage [9]. Free radicals play a crucial role in the pathogenesis of aging, anemia, arthritis, asthma, atherosclerosis, cancer, cardiovascular diseases, diabetes, hypertension, inflammation, myocardial infarction, and neurodegenerative diseases [10]. Naturally all organism possess defense machineries, which are endogenous antioxidants, to guide against the deleterious effect of these reactive oxygen species [11]. However, during oxidative stress these endogenous antioxidants get unbalanced by exogenous and endogenous factors leading to various disease conditions [12]. The excessive production of oxidants have led to increased investigations to identify potential antioxidants from natural products basically from plants [13]. The harmful effects of oxidative stress can be reduced by a constant supply of natural products [14]. It is has been well established that herbal medicines are a safer option for prevention of diseases mediated by oxidative stress [15].

This investigation focuses on evaluating the scavenging activities and antioxidant properties of three different species of Nigeria soybeans.

2. Materials and Methods

2.1 Collection and Extraction of Plant Materials

Three accessions *TGx-1835-10E, TGx-1987-62F and TGx 1951-3F* of soybean were collected at National cereals research institute, Niger state, Nigeria. The accessions were oven dried at 40 ^{o}C for 48 hours to get rid of absorbed moisture and the dry seeds were reduced to fine powder using an electronic blender. Fifty (50) gram of powdered material were subjected to soxhlet extraction using 80% methanol.

2.2 Determination of Total Phenol Content`

The method of Singleton and Rossi [16] was used as described by Gulcin *et al* [17] using the Folin ciocalteu's phenol reagent which is an oxidizing reagent. To a mixture of 0.1 ml of sample and 0.9 ml of distilled water, was added 0.2 ml of Folin-ciocalteu's phenol reagent and the resulting mixture vortexed. After 5 minutes, 1.0 ml of 7% (w/w) Na_2CO_3 solution then added and the solution was then make up to 2.5 ml before incubating for 90 minutes at room temperature. The absorbance against a negative control containing 0.1ml of water in place of the sample was then taken at 750 nm. Gallic acid (0.1mg/ml) was used as standard in order to determine Gallic acid Equivalent (GAE) of sample, after preparing a calibration curve. Distilled water was used as blank.

2.3 Determination of Total Flavonoid Content

Standard quercetin with varying concentration 20, 40, 60, 80 and 100 µg/ml were used as standard. The assay was carried out based on the Aluminium chloride colorimetric assay method according to Zhilen [18] as described by Miliauskas [19]. To 0.1 ml of extract/standard was added 0.4ml of distilled water. This was followed by 0.1ml of 5% sodium nitrite. After 5minutes, 0.1ml of 10% Aluminum Chloride and 0.2ml of sodium hydroxide was added and the volume was made up to 2.5ml with distilled water. The absorbance at 510nm was measured against the blank. The total flavonoid content of the plant, expressed as mg quercetin equivalents per gram of the plant extract is calculated as:

$$X=q^{*}\ Vw$$

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X = Total content of flavonoid compound in quercetin equivalent
q= concentration of quercetin established from the standard curve
V= volume of extract (ml)
w= weight of the crude methanolic extract obtained.

2.4 2, 2-Diphenyl-2-Picryl-Hydrazyl Radical Scavenging Assay

The radical scavenging ability of the samples was determined using the stable radical DPPH (2, 2-diphenyl-2-picryl-hydrazyl hydrate) as described by Brand-Williams [20]. To 1 ml of different concentrations (5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mg/ml) of the extract or standard in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30 minutes after which the absorbance was read at 517 nm against a DPPH control containing only 1 ml methanol in place of the extract.

The percent of inhibition was calculated as follows:
I% = [(Ablank-Asample)/Ablank] x 100
Where A_{blank} is the absorbance of the control (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

2.5 Inhibition of Nitric Oxide (NO) Radical

The nitric oxide scavenging activity of the sample was measured spectrophotometrically according to the method of Green [21] as described by Marcocci *et al*. [22]. The reaction mixture, containing 0.1 ml of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/ml) of the oil extract and 0.9 ml of sodium nitroprusside (2.5 mM) in phosphate buffer saline (pH 7.2, 10 mM) was incubated under illumination for 150 min. After incubation, 0.5 ml of 1% sulphanilamide in 5% phosphoric acid was added and incubated in the dark for 10 min., followed by addition of 0.5 ml 0.1% NED (N-1-napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was measured at 546nm [23]. The percentage inhibition of nitric oxide radical formation was calculated as expressed above in DPPH radical scavenging assay.

2.6 Determination of Total Antioxidant Capacity

This method is based on the reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of a green phosphate/Molybdenum (V) complex at an acidic pH [9]. To 0.1 ml of the extracts (1 mg/ml) or standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/ml) was added 1 ml of the reagent solution which consisted of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes containing the reacting mixture were incubated in a water bath at 95°C for 90 min. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695nm against a blank which consisted of the reacting mixture containing distilled water in place of the extract. The antioxidant activities of the extracts were expressed as an ascorbic acid equivalent.

2.7 Ferric Reducing Antioxidant Power (FRAP)

The FRAP working reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and 20 mM $FeCl_3.6H_2O$ were mixed together in the ratio of 10:1:1 respectively. A 50 µl aliquot of the oil extract at 0.1 mg/ml and 50 µl of standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/ml) were added to 1 ml of FRAP reagent in duplicate tubes. Absorbance measurement was taken at 593nm exactly 10 mins after mixing against reagent blank containing 50 µl of distilled water. All measurements were taken at room

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temperature with samples protected from direct sunlight. The Ferric reducing antioxidant power was expressed in ascorbic acid equivalent concentration (EC) which was defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard [24].

2.8 Ferrous Ion-chelating Ability Assay

The ferrous ion-chelating (FIC) assay was carried out according to the method of Singh and Rajini [25] with some modifications. Solutions of 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine were diluted 20 times. Briefly, an aliquot (1 ml) of different concentrations of extracts were mixed with 1ml $FeCl_2 \cdot 4H_2O$. After 5 min incubation, the reaction was initiated by the addition of ferrozine (1 ml). The mixture was shaken vigorously and after a further 10 min incubation period the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formations was calculated by using the formula:

Chelating effect % = $[(A_{control}-A_{sample})/A_{control}] \times 100$
Where, $A_{control}$ = absorbance of control sample (the control contains FeCl2 and ferrozine, complex formation molecules) and A_{sample} = absorbance of a tested samples.

2.9 CUPRAC Assay

In order to determine the cupric ions (Cu^{2+}) reducing ability of extracts, the method of Apak [26]. was used with little modification as described by Gulcin [27]. Briefly, 0.25 ml $CuCl_2$ solution (0.01M), 0.25 ml ethanolic neocuproine solution (7.5 * 10-3M), and 0.25ml CH_3COOH_4 buffer (1M) were added to a test tube, followed by mixing with 0.25ml of extracts. The total reaction volume was adjusted to 2 ml with distilled water, and the solution was mixed well. The tubes were stoppered and kept at room temperature for 30 min, and absorbance was measured at 450 nm. Increased absorbance indicates increased reduction capability which is express as trolox equivalent (TEAC) using trolox as standard.

3. Statistical and Data Analysis

All data obtained from the various experiment were subjected to descriptive statistical calculation using GraphPad® Instat Statistical Package and expressed as mean values and standard error of mean (S.E.M) of multiple measurements (usually n=3) . The IC_{50}, values were estimated from graphical linear plots. The level of significance was chosen as $p<0.05$. All the graphs were plotted using GraphPad ® Prism 5 Graphical package. The correlation coefficient, slope and intercept were obtained by linear regression analysis.

4. RESULT AND DISCUSSION

Table 1 showed the results of the DPPH inhibitory assays carried out on the three accessions *TGx-1835-10E, TGx-1987-62F and TGx 1951-3F* of soybean TGx 951-3F has the highest DPPH radical scavenging activity with IC_{50} values of 2.61±0.02 mg/ml, compared to TGx-1835-10E and TGx-1987-62F with an IC_{50} of 2.80 ± 0.06 and 3.28 ± 0.05 mg/ml respectively. The radical scavenging activity soybeans exhibit a dose dose/concentration dependent relationship. DPPH has been used to evaluate the free radical- scavenging activity of natural antioxidants. DPPH is a radical that changes into a stable compound by reacting with an antioxidant and the extent of the reaction depends on the hydrogen donating ability of the antioxidant [28]. The ability of soybean accession to scavenge DPPH radicals suggests that it is an electron donor which can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

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The nitric oxide inhibition assay also showed that TGx 1951-3F has the highest activity with IC_{50} value of 2.58 ± 0.02 mg/ml, compared to TGx-1835-10E and TGx-1987-62F with an IC_{50} of 3.77 ± 0.06 and 3.12 ± 0.10 mg/ml respectively as shown in Table 2. Nitric oxide is an important chemical mediator produced by several different types of cells, including endothelial cells, neurons and macrophages. They are involved in the regulation of various physiological processes, for example the early release of nitric oxide through the activity of constitutive nitric-oxide synthase is important in maintaining the dilation of blood vessels. However excess concentration of NO is associated with several oxidative damages (diseases), for example excess NO reacts with oxygen and superoxide radical, forming the highly reactive peroxynitrite anion (ONOO-) [29]. In this study, the extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide, thereby inhibiting the formation of anions. The result shows that the soybean has moderate nitric oxide scavenging activity compared to the standard ascorbic acid.

In the Ferrous Ion-chelating Ability (FIC) assay, the result in Table 3 shows that the TGx 1951-3F show the highest FIC activity with the least IC_{50} value of 1.38 ± 0.07 mg/ml, compared to TGx-1835-10E and TGx-1987-62F with an IC_{50} of 1.86 ± 0.16 and 2.07 ± 0.16 mg/ml. In metal chelating assay, TGx 1951-3F has the higher ability to chelate metals followed by TGx-1835-10E and TGx-1987-62F. Free iron plays an important role in formation of reactive oxygen species [30]. In addition, excessive iron deposition in different vital organs can lead to the loss of function of those organs like liver, kidney etc. So, chelation of this free iron can prevent the formation of free-radicals as well as can prevent the damage of this vital organ. Ferrozine in complex with ferrous ion (Fe^{2+}) produces a violet colour. In the presence of a chelating agent, complex formation is interrupted by competing with ferrozine in chelating Fe^{2+} and as a result the violet color of the complex is decreased. In this study, the results demonstrated that formation of the ferrozine-Fe^{2+} complex is interrupted in the presence of the soybean accession and standard EDTA.

The ability of the accessions to act as reducing agent was also evaluated using FRAP, TAC and CUPRAC assays. The results show that TGx 1951-3F has highest reducing property follow by TGx-1835-10E and TGx-1987-62F in terms of ascorbic acid equivalent (AAE) of the three samples Table 4. This same trend was also observe in antioxidant constituent present in the samples as TGx-1835-10E has higher phenolic and flavonoid content compared to TGx-1835-10E and TGx-1987-62F as shown in Table 5.

Table 1: **DPPH Radical Scavenging Activity of *TGx-1835-10E, TGx-1987-62F and TGx***

Concentration (mg/mL)	**% Inhibition ± S.E.M**		
	TGx-1835-10E	*TGx-1987-62F*	*TGx 1951-3F*
5	72.03 ± 1.13	67.57 ± 0.76	82.82 ± 0.42
2.5	56.14 ± 0.69	43.28 ± 0.79	50.71 ± 0.23
1.25	37.34 ± 1.46	28.88 ± 0.63	37.08 ± 0.23
0.625	21.32 ± 1.19	21.64 ± 0.78	18.28 ± 0.62
0.3125	12.53 ± 1.61	18.48 ± 0.91	15.57 ± 0.32
0.15625	8.33 ± 0.21	16.54 ± 0.93	12.98 ± 0.27
0.078125	4.97 ± 0.37	12.86 ± 0.14	12.98 ± 2.18
IC50	2.80 ± 0.06	3.28 ± 0.05	2.61 ± 0.02

All analyses are the mean of triplicate measurements ± standard error of mean

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Table 2: **Nitric Oxide Scavenging Activity of *TGx-1835-10E, TGx-1987-62F and TGx***

Concentration (mg/mL)	**% Inhibition ± S.E.M**		
	TGx-1835-10E	*TGx-1987-62F*	*TGx 1951-3F*
5	37.28 ± 1.76	44.30 ± 1.63	63.19 ± 0.73
2.5	31.75 ± 0.28	38.85 ± 0.76	52.96 ± 0.25
1.25	28.62 ± 0.18	28.44 ± 2.62	43.34 ± 0.20
0.625	21.17 ± 0.20	21.77 ± 2.34	40.16 ± 0.28
0.3125	10.44 ± 1.08	8.55 ± 0.91	35.64 ± 1.06
0.15625	2.71 ± 1.76	5.99 ± 0.81	28.87 ± 0.30
IC50	3.77 ± 0.06	3.12 ± 0.10	2.58 ± 0.004

All analyses are the mean of triplicate measurements ± standard error of mean

Table 3: **Metal Chelating Activity of *TGx-1835-10E, TGx-1987-62F and TGx***

Concentration (mg/mL)	**% Inhibition ± S.E.M**		
	TGx-1835-10E	*TGx-1987-62F*	*TGx 1951-3F*
5	88.98 ± 2.41	85.07 ± 0.20	85.57 ± 0.84
2.5	74.34 ± 1.22	73.70 ± 3.67	84.86 ± 1.31
1.25	46.41 ± 6.27	48.40 ± 4.82	60.20 ± 6.54
0.625	30.06 ± 1.75	21.77 ± 2.34	20.04 ± 5.08
0.3125	14.07 ± 3.60	8.55 ± 0.91	7.11 ± 0.35
IC50	1.86 ± 0.12	2.07 ± 0.16	1.38 ± 0.07

All analyses are the mean of triplicate measurements ± standard error of mean

Table 4: **Total Antioxidant Capacity, CUPRAC and FRAP of *TGx-1835-10E, TGx-1987-62F and TGx***

Accessions	**Total Antioxidant Capacity**	**FRAP**	**CUPRAC**
	µg AAE/g ± S.E.M.		
TGx-1835-10E	6.21 ± 0.05	3.12 ± 0.20	29.90 ± 2.37
TGx-1987-62F	1.56± 0.41	1.08 ± 0.17	21.55 ± 1.21
TGx 1951-3F	15.48±0.94	3.66 ± 0.14	34.62 ± 0.30

All analyses are the mean of triplicate measurements ± standard error of mean; TAC, CUPRAC and FRAP were Expressed as mg Ascorbic acid Equivalent/g of dry plant material.

Table 5: **Total Phenol and Flavonoids Contents of *TGx-1835-10E, TGx-1987-62F and TGx***

Accessions	**Total Phenol Content µg GAE/g ± S.E.M.**	**Total Flavonoid Content µg QUE/g± S.E.M.**
TGx-1835-10E	75.33±0.24	8.65 ± 0.30
TGx-1987-62F	55.55± 0.11	8.04 ± 0.66
TGx 1951-3F	82.91 ± 0.05	13.21 ± 1.79

All analyses are the mean of triplicate measurements ± standard error of mean; TFC: Expressed as mg quercetin Equivalent/g of dry plant material; TPC: Expressed as mg Gallic acid Equivalent /g of dry plant material

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Plant phenolics are known to exhibit potent antioxidant activity [31]. Also, the anti-oxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, and inhibition of enzymes responsible for free radical generation [32]. Hence, the observed antioxidant activity of the extracts of soybean accession may be due to the presence of these constituents. However, variations in activity of different accessions may be due to the diversity in the basic chemical structure of phyto-constituents, which make them, possesses different degree of antioxidant activity against different free radicals.

5. CONCLUSION

The result of this study revealed that the three accessions TGx-1835-10E, TGx-1987-62F and TGx 1951-3F of soybean, contains huge amounts of flavonoids and phenolic compounds, exhibits high antioxidant and free radical scavenging activities. This important evident suggests that the three accessions have great health benefit.

CONSENT (WHERE EVER APPLICABLE)

Not applicable

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