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Aqueous extract of *Solanum anguivi* Lam. fruits (African Egg Plant) inhibit Fe^{2+} and SNP induced lipid peroxidation in Rat's brain – *In Vitro*

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ABSTRACT

The ability of the aqueous extract of flesh and seed of Solanum anguivi Lam fruit (African eggplant) used in folk medicine to treat diabetes and hypertension to inhibit Fe^{2+} and SNP induced lipid peroxidation in rats brain in vitro was determined. The total phenol, total flavonoid, vitamin C content, ferric reducing antioxidant property as well as Fe^{2+} chelating ability were also determined. The results of this study revealed that aqueous extract of Solanum anguivi fruit (flesh and seed) cause a significant decrease ($P<0.05$) in the MDA production in rats brain when compared with control. The inhibitory effect of both flesh and seeds of Solanum anguivi on lipid peroxidation could be attributed to the phenol (flesh, 7.13 ± 0.73mg/g GAE, seed 8.20 ± 0.32 mg/g GAE), total flavonoid (flesh 3.53 ± 0.49mg/g QE, seed 1.62 ± 0.61mg/g QE) and vitamin C content (flesh 12.85 ± 0.20mg/g, seed 14.92 ± 0.11 mg/g), as well as ferric reducing antioxidant property and Fe^{2+}- chelating ability. Hence, Solanum anguivi Lam. fruit could be used in the management of type 2 diabetes complication due to its antioxidant ability, a property that play a major role in ameliorating complications resulting from oxidative damage in diabetes.

Key words: Solanum anguivi Lam. fruit, antioxidant properties, lipid peroxidation, African eggplant, Phytochemicals, medicinal plants.

INTRODUCTION

Plants have been a source of medicine in the past centuries and today scientists and the general public recognize their value as a source of new or complimentary medicinal products. Recently, wide array of research investigations highlight the potential health benefits from plant sources. Over the past twenty years, interest in medicinal plants has grown enormously from the use of herbal products as natural cosmetics and for self-medication by the general public to the scientific investigations of plants for their biological effects in human beings. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal product to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people [1].

Several researchers considered brain to be abnormally sensitive to oxidative damage and many studies have demonstrated the ease of peroxidation of brain membranes [2, 3, 4, 5]. Brain contains high level of fatty acids which are more susceptible to peroxidation, which consumes an inordinate fraction (20%) of total oxygen consumption for its relatively small weight (2%). In addition, it is not particularly enriched in antioxidant defense. Brain is lower in antioxidant activity in comparison with other tissues, for example, about 10% of liver. Moreover, human brain has

higher level of iron in certain regions and in general has high levels of ascorbate [6, 7] Iron (Fe) is an essential metal for normal cellular physiology and plays a catalytic role in the initiation of free radical reactions. Fe is also necessary in relatively large amounts for hemoglobin, myoglobin and cytochrome production, xanthine oxidase but other Fe proteins require rather small amounts of Fe. On the other hand, free Fe in the cytosol and in the mitochondria can cause considerable oxidative damage by increasing superoxide production [8, 9]. The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates; the result is wide-ranging impairment in cellular function and integrity [10]. The mechanism by which iron can cause this deleterious effect is that Fe^{2+} can react with hydrogen peroxide (H_2O_2) to produce the hydroxyl radical ($OH^{\cdot}$) via the Fenton reaction, whereas superoxide can react with Fe^{3+} to regenerate Fe^{2+} that can participate in the Fenton reaction [8,9]. The overproduction of ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. The inhibition of ion pump ATPases in plasma membranes can also occur as a result of iron-promoted formation of ROS and subsequent lipid peroxidation. Moreover, the reactive oxygen species can activate the transcription factors as nuclear factor-kB (NF-kB), which up-regulates the transcription of adhesion molecules, cytokines and enzymes, all involved in the inflammatory responses [11].

Antioxidants are considered as possible protection agents reducing oxidative damage of human body from ROS and retard the progress of many chronic diseases as well as lipid peroxidation [12].Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of free radical mediated diseases. The development of safer natural antioxidants from extracts of spices and other plant materials that can replace synthetic antioxidants is of interest. Nowadays, natural antioxidants have become a major area of scientific research [13, 14]; therefore, the importance of searching for and exploiting natural antioxidants, especially those of plant origin, has increased greatly in recent years.

Solanum anguivi belong to the family *Solanaceae* and can be found throughout the non- arid parts of Africa [15]. *S. anguivi* have been recognized to possess medicinal properties and their use in traditional systems of medicine has been on record for a long time. It is a nourishing vegetable commonly used in soup and medically to control high blood pressure and diabetes [16].

Although, reports abound on biological activities of *Solanum anguivi* fruits like, cholesterol lowering activity, [17], hypolipidemic property [18], *in vivo* antioxidant activity [19], there are dearth of information on the neuroprotective potentials of *Solanum anguivi* fruits, therefore the need to determine the neuroprotective potentials of *Solanum anguivi* fruit (flesh and seed), as well as estimate the phenolic content and evaluate their antioxidant activities.

MATERIAL AND METHODS

Chemical reagents

Thiobarbituric acid (TBA) was obtained from Sigma (St. Louis, MO). 2, 2-diphenyl -1-picrylhydrazyl (DPPH) and 1, 10 phenanthroline were obtained from Fluka Chemie and Merck (Germany). All other chemicals were obtained from standard chemical suppliers and were of analytical grade.

Sample collection:

Fruits of *solanum anguivi* were obtained from a farm in Akungba Akoko, Ondo State Nigeria and were taken to Department of Plant Science and Biotechnology, Adekunle Ajasin University for identification and authentication. The seeds were carefully removed from the flesh (skin) and were then sun-dried before they were powdered and kept in an airtight container prior to analysis.

Handling and use of animal

The handling and the use of the animals were in accordance with NIH Guide for the care and use of laboratory animals. In the experiment, Wister strain albino rats weighing 200–230 g were purchased from the breeding colony of Department of Veterinary Medicine, University of Ibadan, Nigeria. Rats were maintained at 25oC, on a 12 h light/12 h dark cycle, with free access to food and water. They were acclimatized under these conditions for two weeks before the experiment.

Aqueous extract preparation

Five grams each of flesh and seed of *S. anguivi* fruit were weighed in two separate extraction bottle and one hundred milliliters of distilled water was added to the bottles containing the flesh and seed respectively and left for

24 h to allow for extraction. Thereafter, the solutions were filtered separately using a Whatman filter paper. The extracts were stored air tight in a refrigerator until required for use. This serves as the stock solution for all determinations.

Lipid peroxidation assay

Rats were decapitated under mild ether anesthesia and the cerebral (whole brain) was rapidly dissected, placed on ice and weighed. The brain tissue was immediately homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, w/v). The homogenates were centrifuged for 10 min at 4000 g to yield a pellet that was discarded and a low-speed supernatant (S1). An aliquot of 100 µl of S1 was incubated for 1 h at 37°C in the presence of both walnut extracts, with and without the prooxidants, iron (final concentration (10 µM)) and sodium nitroprusside (SNP) (final concentration 30 µM). This was then used for lipid peroxidation determination. Production of thiobarbituric acid reactive species. (TBARS) was determined as described by [20]. The color reaction was developed by adding 300 ml 8.1% sodium dodecyl sulfate (SDS) to S1, followed by sequential addition of 500 ml acetic acid/HCl (pH 3.4) and 500 ml 0.8% thiobarbituric acid (TBA). This mixture was incubated at 95°C for 1 h. TB ARS produced were measured at 532 nm and the absorbance was compared to that of the controls.

Determination of vitamin C content

Vitamin C content of both seed and flesh were determined using the method of [21]. Briefly, 75µL DNPH (2 g dinitrophenyl hydrazine, 230mg thio urea and 270mg CuSO4.5H2O in 100mL of 5mol L-1 H_2SO_4) were added to 500µL reaction mixture (300µL of an appropriate dilution of the polar extract with 100µL 13.3% (TCA) and water).The reaction mixtures were subsequently incubated for 3 h at 37^o C, then 0.5mL of 65% H2SO4 (v/v) was added to the medium; their absorbance was measured at 520nm and the vitamin C content of the samples was subsequently estimated from standard ascorbic acid.

Determination of total phenol content

The total phenol content of both extracts were determined by the method reported by [22]. Appropriate dilutions of the extracts were oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45^o C and the absorbance was measured at 765 nm with a spectrophotometer. The total phenol content was subsequently calculated and reported in percentage (%). Gallic acid was used as standard phenol.

Determination of total flavonoid content

The total flavonoid content of both extracts was determined using a slightly modified method reported by [23]. Briefly, 0.5 ml of appropriately diluted sample was mixed with 50 µl of 10% AlCl3, 50 µl of potassium acetate and 1.4 ml water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard.

Fe^{2+} chelation assay

The Fe2+ chelating ability of the spice extracts was determined by method of Minotti with slight modification by [24]. Freshly prepared 500µM FeSO4 (150µL) was added to a reaction mixture containing 168µL of 0.1M Tris-HCl (pH 7.4), 218µL of saline and the extract (25µL). The reaction mixture was incubated for 5 min, followed by the addition of 13µL of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm with a spectrophotometer. The Fe2+ chelating ability was subsequently calculated with respect to the reference, which contains all the reagents without the test sample.

Determination of reducing property

The reducing properties of the extracts were determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by [25]. 2.5ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50 ^{o} C for 20 min. and then 2.5 ml 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1 % ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated.

Deoxyribose degradation

Deoxyribose degradation was determined by [26]. Deoxyribose is degraded by hydroxyl radicals with the release of thiobarbituric acid (TBA) reactive materials. Deoxyribose (6 mM) was incubated at 37°C for 30 min with 50 mM

potassium phosphate pH 7.4 plus Fe2+ (0.1 mM) and/or H2O2 (1 mM) to induce deoxyribose degradation, and seed extracts (0 - 50 µl of stock). After incubation, 0.4 ml of TBA 0.8% and 0.8 ml of TCA 2.8% were added, and the tubes were heated for 20 min at 100°C and spectrophotometrically measured at 532 nm.

Data Analysis

The result of three replicate experiments were pooled and expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was carried out. Significance was accepted at $P<0.05$.

RESULTS AND DISCUSSION

Effect of aqueous extract of flesh and seed of *S. anguivi* fruit on lipid peroxidation induced by Fe^{2+} and SNP in rat brain homogenate.

Fig. 1 and 2 show the effect of aqueous extract of flesh (F) and seed (S) of *S. anguivi* fruit on the Fe^{2+} and SNP induced formation of TBARS from brain homogenate respectively. The results clearly show that incubation of the rats brain with 10µM Fe^{2+} (fig. 1) and 3µM SNP (fig.2) in the presence of aqueous extract of flesh (F) and seed (seed) of *S. anguivi* exerted a significant inhibitory effect on the peroxidation process with the flesh having the higher inhibitory effect than seed in Fe^{2+} induced peroxidation while the seed exhibited higher inhibitory effect in SNP induced oxidative assault.

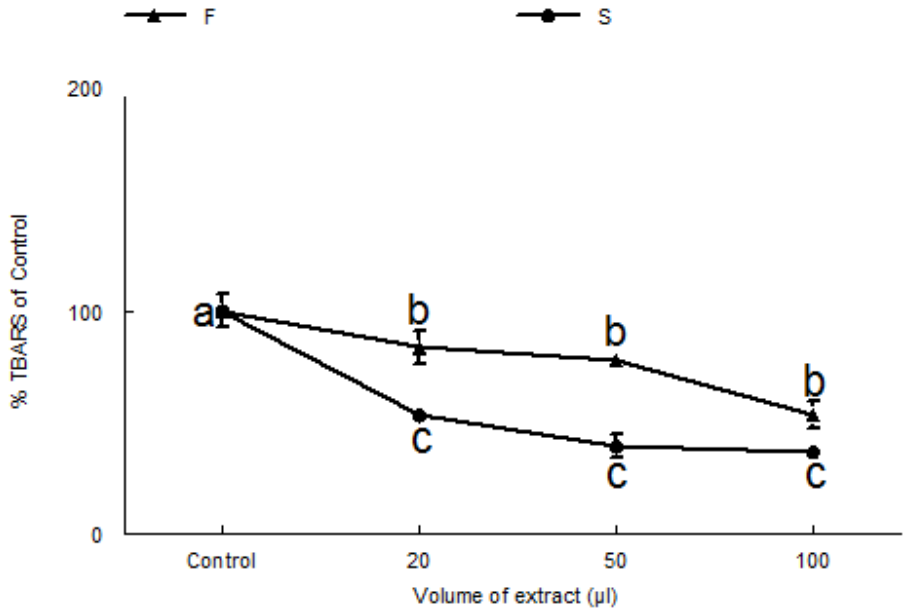

Figure 1. Inhibition of Fe2+ induced cerebral lipid peroxidation by aqueous extract of flesh (F) and seed (S) of *S. anguivi* fruit. Values are given as mean ± SEM of 3 – 4 independent experiment performed in triplicate "b" and "c" indicate a significant difference from control "a" at (P<0.05).

The antioxidant constituents of *S. anguivi* fruit (Flesh and seed) that was determined in the present study as shown in Table 1 include total phenols, flavonoids and vitamin C. The phenolic content of the fruit of S. anguivi was estimated to be flesh 7.13 ± 0.73mg/g (GAE), seed 8.20 ± 0.32 mg/g (GAE). Whereas, flavonoid content was estimated to be 3.53 ± 0.49mg/g (QE) for flesh and 1.62 ± 0.61mg/g (QE) for seed. In addition, vitamin C content was 12.85 ± 0.20mg/g for flesh and 14.92 ± 0.11mg/g for seed. GAE denotes gallic acid equivalent while QE denotes quercetin equivalent.

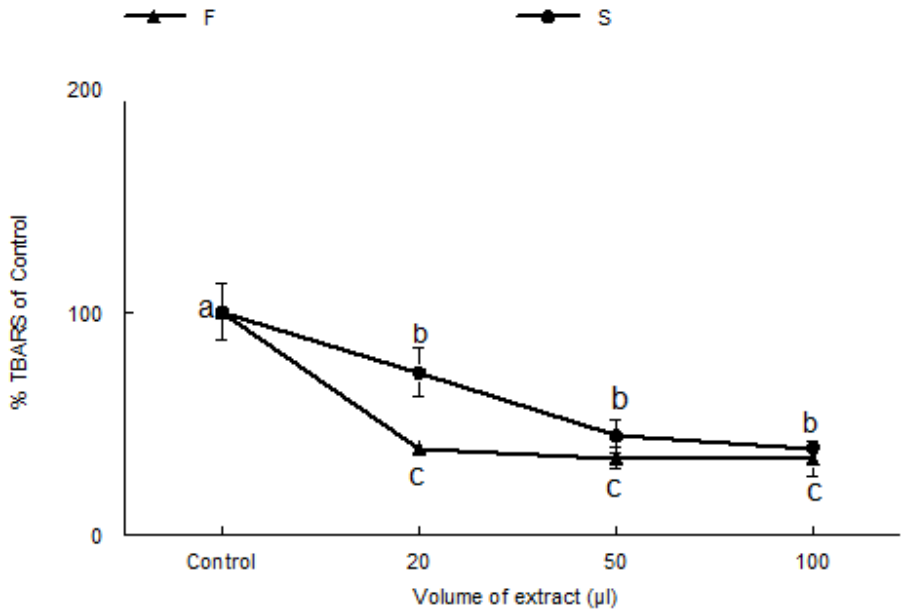

Figure 2. Inhibition of SNP induced cerebral lipid peroxidation by aqueous extract of flesh (F) and seed (S) of *S. anguivi* fruit. Values are given as mean ± SEM of 3 – 4 independent experiment performed in triplicate "b" and "c" indicate a significant difference from control "a" at (P<0.05).

Table1. Antioxidant constituents of aqueous extract of flesh and seed of *S. anguivi* fruit

Sample	Phenol mg/g GAE	Flavonoid mg/g QE	Vitamin C mg/g
Flesh (F)	7.13 ± 0.73	3.53 ± 0.49	12.85 ± 0.20
Seed (S)	8.20 ± 0.32	1.62 ± 0.61	14.92 ± 0.11

Values represent mean ± standard deviation, n = 3

Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and a biotic stress condition such as infection, water stress, and cold stress [27] and can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure [28] Polyphenols are common constituents of the human diet, present in most foods and beverages of plant origin. They are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases [29]. They are considered to contribute to the prevention of various degenerative human diseases such as Alzheimer's diseases. This assumption originally came from in vitro studies, showing the antioxidant properties of polyphenols and their ability to modulate the activity of various enzymes. The antioxidant constituents of aqueous extract of flesh and seed of *S. anguivi* fruit is also presented in Table 1. The total phenol content of the flesh 7.13 ± 0.73mg/g, seed 8.20 ± 0.32 mg/g. and flavonoid content 12.85 ± 0.20mg/g for flesh and 14.92 ± 0.11mg/g for seed. Both flesh ad seed have higher flavonoid content than phenol.

Vitamin C plays an important role in the in the regulation of various neurotransmitters, including nor epinephrine and acetylcholine which are critical for brain function. Vitamin C is a water soluble vitamin which has been known to protect essential substances in the body such as proteins, lipids, carbohydrates and DNA and RNA from damage by free radicals [30,31]. The result of the vitamin C content of the fruit of *S. anguivi* is presented in Table 1. Vitamin C content of flesh 12.85 ± 0.20mg/g and 14.92 ± 0.11mg/g for seed with the seed having higher vitamin C content than flesh.

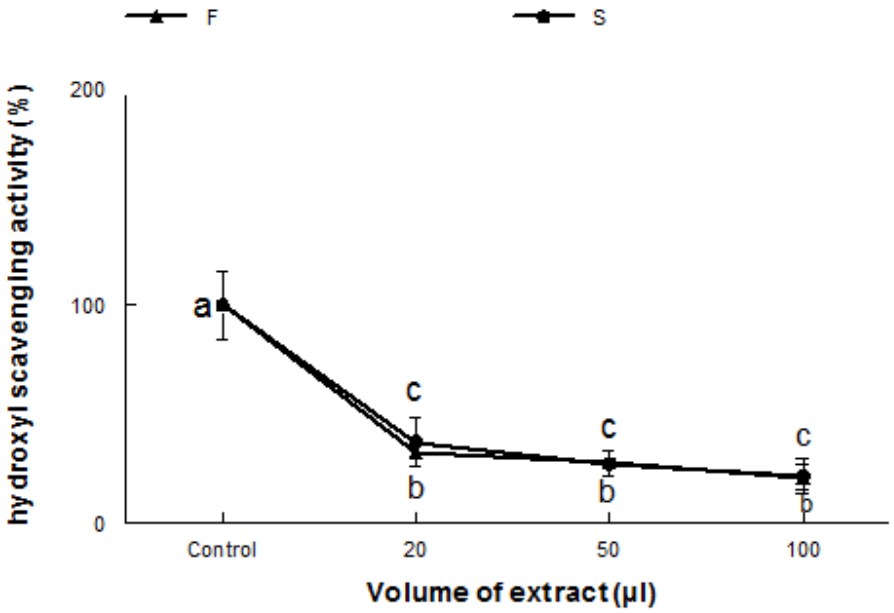

Figure 3. Inhibition of deoxyribose degradation by aqueous extract of flesh (F) and seed (S) of *S. anguivi* fruit. Values are given as mean ± SEM of 3 – 4 independent experiment performed in triplicate "b" and "c" indicate a significant difference from control "a" at (P<0.05).

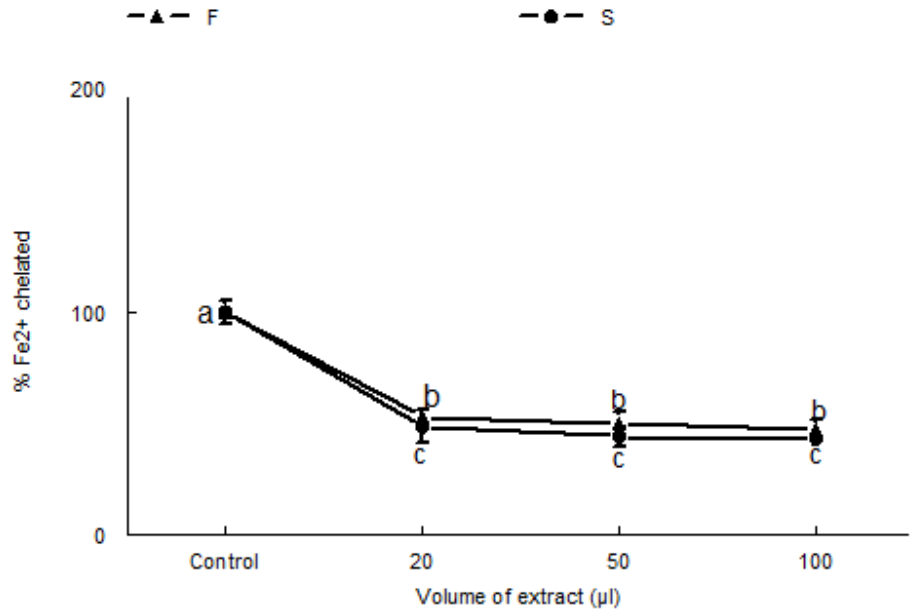

Figure 4. Iron chelating property of aqueous extract of flesh (F) and seed (S) of *S. anguivi* fruit. Values are given as mean ± SEM of 3 – 4 independent experiment performed in triplicate "b" and "c" indicate a significant difference from control "a" at (P<0.05).

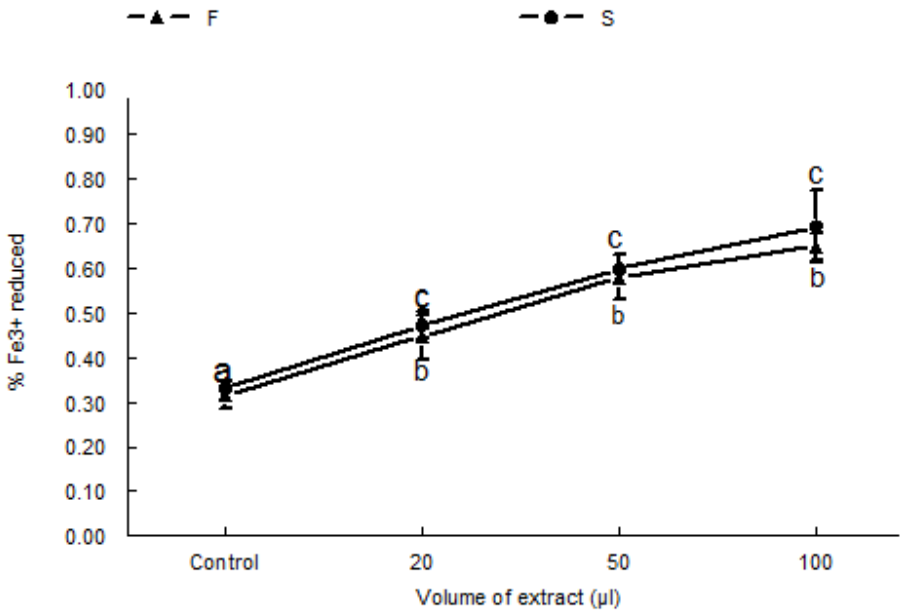

Figure 5. Reducing property of aqueous extract of flesh (F) and seed (S) of *S. anguivi* fruit. Values are given as mean ± SEM of 3 – 4 independent experiment performed in triplicate "b" and "c" indicate a significant difference from control "a" at (P<0.05).

The total antioxidant capacity is a summation of different antioxidant mechanisms, including free radical scavenging ability, reducing power and Fe^{2+} chelating ability. Hence, the decrease in the Fe^{2+} -induced lipid peroxidation in the Rat's brain homogenates in the presence of the extracts could be as a result of the ability of the antioxidant phytochemicals in flesh and seed extracts to chelate Fe^{2+} and scavenge free radicals produced by the Fe^{2+} catalyzed production of reactive oxygen species (ROS) in the Rat's brain homogenates [30,31]. In order to provide an explanation for the inhibition of Fe^{2+} -induced lipid peroxidation in Rat's brain tissue, ferric reducing antioxidant property, Fe^{2+}- chelating ability and deoxyribose inhibitory properties of the water extractable phytochemicals in the *Solanum anguivi* fruit were determined and the results are shown in Figure 5, 4, and 3. Ferric reducing antioxidant property (FRAP) is a novel anti oxidation defense mechanism; the two mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer [32]. The reducing power of *Solanum anguivi* fruit extracts was assessed based on their ability to reduce Fe (III) to Fe (II) and the results are presented in Figure 3. Seed has higher iron reducing property indicating that fruit of *Solanum anguivi* is rich in free electrons and readily supply such electron to Fe3+ thereby reducing it to Fe^{2+}. The reducing ability was significant at the least volume of extract tested.

The ability of antioxidants to chelate and deactivate transition metals and prevents such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal-catalyzed reaction is considered to be due to an antioxidant mechanism. Fe^{2+} chelating ability of the *Solanum anguivi* fruit extracts was determined and the result is presented in Figure 4. The result revealed the Fe^{2+} chelating property of flesh and seed of *Solanum anguivi* fruit with seed having higher chelating effect than flesh.

The Fenton process is based on an electron transfer between H_2O_2 and Fe^{2+} acting as homogenous catalyst. Hydroxyl radicals (OH**.**) are generated during Fe^{2+} - catalyzed decomposition of hydrogen peroxide [33]. Figure 3 shows the inhibition of deoxyribose degradation by flesh and seed of *S. anguivi*. The flesh has higher inhibitory effect than the seed.

The result reveals that flesh and seed extract of *Solanum anguivi* fruit are rich in vitamin C, phenol as well as flavonoid. Both exhibit Fe^{2+}- chelating ability, ferric reducing antioxidant property and inhibition of Fe^{2+}/H_2O_2-induced decomposition of deoxyribose. Therefore, phenols, flavonoids, and vitamin C may be actively involved in the protection of brain tissue from Fe^{2+} and SNP induced lipid peroxidation, the mechanism through which they

possibly do this, is by their ferric reducing antioxidant property, Fe^{2+} chelating ability, and inhibition of Fe^{2+}/H_2O_2-induced decomposition of deoxyribose.

This result reveals that aqueous extract of flesh and seed of *Solanum anguivi* fruits have a protective effect against lipid peroxidation formation in brain of albino rats *in vitro*.

Also, phenols, flavonoids, and vitamin C may be actively involved in the protection of brain tissue from Fe^{2+} and SNP induced lipid peroxidation, the mechanism through which they possibly do this, is by their ferric reducing antioxidant property, Fe (II) chelating ability, and inhibition of $Fe^{2+/}H_2O_2$- induced decomposition of deoxyribose. This property of Solanum anguivi may be responsible for its use in the treatment of diabetes as widely claimed in folk medicine.

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