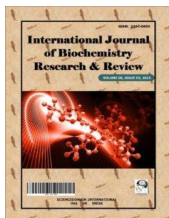

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In vitro Antioxidant Capabilities and Vitamin Levels in Beef, Chicken and Fish Homogenates

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FMA and WI designed the study, performed the statistical analysis, wrote the protocol, author WI wrote the first draft of the manuscript. Author ADJ managed the analyses of the study. Authors KMS, IA and CAU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In this modern era of functional food research, there are possibilities that we could eat not only to derive nutritional values but also to get well. In this study, the antioxidant and vitamin level in fish, beef and chicken homogenates were determined using various assays. The results showed that vitamin E levels in fish homogenate (34.47 ± 1.04 µg/dL) were significantly high ($p<0.05$) compared with chicken homogenate (29.55 ± 4.84 µg\dL), and beef homogenate (19.16 ± 0.96 µg/dL). Also the vitamin C levels in the beef homogenates (51.12 ± 3.74) was significantly higher ($p<0.05$) compared to the chicken homogenates (28.60 ± 2.42 mg/dL) and fish homogenates (26.39 ± 1.63). However, there was no significant difference ($p>0.05$) in the vitamin C levels between the fish and chicken homogenates. The fish and chicken homogenate showed significant dose-dependent DPPH radical inhibiting capacities, inhibiting 17.55 ± 3.71% and 16.61 ± 2.22% of DPPH at a concentration of 15 mg/mL compared to the meat homogenate which inhibited 11.68 ± 1.78% at the same

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concentration. The IC50 of superoxide anion radical inhibition was found to be significantly lower ($p<0.05$) for the chicken and meat homogenate compared to the fish homogenate. From this experiment it can be postulated that fish and beef have a higher antioxidant levels compared to chicken and could be better source of functional foods.

Keywords: Antioxidant; vitamin E; DPPH; vitamin C.

1. INTRODUCTION

Oxidative damage occurs in the living animal due to an imbalance between the production of reactive oxygen or nitrogen species and the defense mechanism of the animal against oxidative stress. Oxidation is inherent to metabolism, but an excessive formation of reactive species in oxidation processes can cause damage to vital components in biological systems [1].

Oxidation increases as a result of a high intake of oxidized lipids, oxidation of sensitive polyunsaturated fatty acids (PUFA), pro oxidants, or a low intake of nutrients involved in the antioxidant defense system [2]. Oxidation is a very general process, which affects lipids, pigments, proteins, DNA, carbohydrates, and vitamins [3].

Oxidation limits storage time and thereby also affects marketing and distribution of both fish and Beef products. Especially fish, being rich in n-3 polyunsaturated fatty acids (PUFA) is susceptible to peroxidation of PUFA resulting in restriction of storage and processing possibilities [4]. Furthermore, peroxidative products, particularly aldehydes, can react with specific amino acids to form carbonyls and protein aggregates causing additional nutritional losses [5].

In muscle and fat tissue, oxidation continues postmortem and affects the shelf-life of Beef and Beef products. Oxidation can cause quality loss of nutrient in Beef and especially in fish [6]. Lipid oxidation in Beef and fish-products leads to rancid taste and off flavor and development of many different substances from which some have even adverse effects to human health [7].

Beef is animal flesh that is eaten as food [8]. Humans have hunted and killed animals for Beef since prehistoric time. The advent of civilization allowed the domestication of animals such as chicken, sheep, pigs, and cattle and eventually their use in Beef production on an industrial scale.

Beef is mainly composed of water, protein, and fat, and is usually eaten together with other food. It is edible raw, but is normally eaten after it has been cooked and seasoned or processed in a variety of ways.

2. DESCRIPTION OF ANIMAL TISSUES

2.1 Chicken

Broiler chickens (*Gallusgallus domesticus*) are a gallinaceous domesticated fowl, bred and raised specifically for Beef production [9]. They are hybrid of egg-laying chicken, both being a subspecies of red jungle fowl (*Gallusgallus*). Chickens are one of the most common and widespread domestic animals.

Common Names for Chicken:

English : Chicken
Igbo : Anu Okuko
Yoruba : Eran Adiye
Hausa : Naman Kaza

Scientific names:

Kingdom : *Animalia*
Phylum : *Chordata*
Class : *Aves*
Order : *Galliforme*
Family : *Phasianidae*
Sub family : *Phasianinae*
Genus : *Gallus*
Species : *G.gallus*
Sub species : *G. g. domesticus*
Trinomial name : *Gallusgallus domesticus*

Fig. 1. *Gallusgallus domesticus*

2.2 Beef

Cattle also called cows are the most common type of large domesticated ungulates. They are a prominent modern member of the subfamily Bovinae, are the most widespread species of the genus *Bos*, and are most commonly classified collectively as *Bos taurus*. Cattle are raised as livestock for Beef (beef and veal), as dairy animals for milk and other dairy products, and as draft animals (oxen or bullocks that pull carts, plows and other implements). Other products include leather and dung for manure or fuel. In some regions, such as parts of India, cattle have significant religious meaning. From as few as 80 progenitors domesticated in southeast Turkey about 10-500 years ago according to an estimate from 2003, there are 1.3 billion cattle in the world [10].

Common names of cow beef

English : Cow beef
Igbo : Anu afe
Yoruba : Eran malu
Hausa : Naman shanu

Scientific classification

Kingdom : *Animalia*
Phylum : *Chordata*
Class : *Mammalia*
Subclass : *Theria*
Infra class : *Eutheria*
Order : *Cetartiodactyla*
Family : *Bovidae*
Subfamily : *Bovinae*
Genus : *Bos*
Species : *B. taurus*
Binomial name : *Bostaurus*

Fig. 2. Raw meat/beef

2.3 Fish

The West African Spanish mackerel (*Scomberomorus tritor*) is a species of fish in the family Scombridae.

Specimens have been recorded at up to 100 cm in length, and weighing up to 6 kg. Coloration is bluish-green on the back fading to silvery on the sides marked with about 3 rows of vertically elongate orange spots.

It is found in the eatern Atlantic, along the Atlantic coasts of Africa from Canary Island and Senegal to the Gulf of Guinea and Baisa dos Tigres, Angola. It is rarely found in the northern Mediterranean Sea, along the coast of France and Italy [11].

Common names of fish

English : Fish
Local name : Skina and Tuna
Igbo : Azu
Yoruba : Eja
Hausa : Kifi

Scientific Name:

Kingdom : *Animalia*
Phylum : *Chordata*
Class : *Actinoptrygii*
Order : *Perciformes*
Family : *Scombridae*
Genus : *Scomberomonis*
Specie : *S. tritor*
Binomial name : *Scomberomorus tritors*

Fig. 3. Raw fish

Free Radicals and Reactive Oxygen Species: Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body [12].

Free radical is an atom or molecule that has unpaired valence electrons these unpaired electrons make free radicals highly chemically reactive towards other substances. Reactive oxygen species (ROS) are chemically reactive chemical species containing oxygen. Examples include peroxides, superoxide, hydroxyl radical, and singlet oxygen. ROS are formed as a natural byproduct of the normal

metabolism of oxygen, during times of environmental stress UV or heat exposure [13]. ROS are also generated by exogenous sources such as ionizing radiation [14].

Exogenous ROS: Exogenous ROS can be produced from pollutants, tobacco, smoke, drugs, xenobiotics, or radiation. Ionizing radiation can generate damaging intermediates through the interaction with water, a process termed radiolysis. Since water comprises 55–60% of the human body, the probability of radiolysis is quite high under the presence of ionizing radiation. In the process, water loses an electron and becomes highly reactive.

Then through a three step chain reaction, water is sequentially converted to hydroxyl radical($^{\bullet}OH$), hydrogenperoxide(H_2O_2), superoxide radical($^{\bullet}O_2$) and ultimately oxygen(O_2).The hydroxyl radical is extremely reactive and immediately removes electrons from any molecule in its path, turning that molecule into a free radical and thus propagating a chain reaction. However, hydrogen peroxide is actually more damaging to DNA than the hydroxyl radical, since the lower reactivity of hydrogen peroxide provides enough time for the molecule to travel into the nucleus of the cell, subsequently wreaking havoc on macromolecules such as DNA [15].

3. MATERIALS AND METHODS

3.1 Preparation of Beef, Fish and Chicken Homogenates

The samples used for the experiment was purchased from central market in Madonna university Elele. The sample was weighted using weighing balance, homogenized using laboratory mortar and pestle and put inside a beaker and added 100ml of buffer, then refrigerated for 24hr. The samples had the various weights:

Fish - 117.794 g
Beef (beef) - 37.794 g
Chicken - 37.278 g

Then homogenate:

Beef +100 mL of buffer = 229 g
Chicken +100 mL of buffer = 229 g
Fish +100 mL of buffer = 239 g

Samples without buffer solution:

Fish minus buffer = 26.24 g
Beef minus buffer = 24 g
Chicken minus buffer = 24.16 g

3.2 Serial Dilution of Homogenate and Assay Reagent

The animal tissues used for the experiment is one found commonly in Madonna university, a brief selection of three animal tissues was made. The animal tissues are chicken, beef/meat, and fish. Serial dilution was done for each animal tissue homogenate consisting of 10 test tubes including blank and control for various radical scavenging assays.

3.2.1 Ascorbic acid assay

The tissue homogenate concentrations of ascorbic acid were determined spectrophotometrically according to the method of [16]. The principle of the method involves oxidizing ascorbic acid and converting it to diketoglucuronic acid in strong acid solution. A diphenylhydrazone is formed after reacting with 2,4-dinitrophenylhydrazine using cupric ion as an oxidizing agent. Hydrazone dissolves in strong sulphuric acid solution to produce a light red coloration, which can be measured spectrophotometrically at 500 nm. This method measured both ascorbic acid and dehydroascorbic acid. However insignificant dehydroascorbic acid exists in blood and the method produces a substantially accurate result. The coloring agents act specifically by preventing interference to ascorbate chromogens. Plasma ascorbic acid concentrations were determined by mixing 0.5 mL of plasma/serum/homogenate with 0.5mL of 10% trichloroacetic acid and 0.25mL chloroform. The mixtures were stopped and shaken vigorously for about 10-15 seconds with vortex mixer and centrifuged at 5000rpm for 15minutes. Blank and standard were prepared by adding 0.5mL of 10% TCA to 0.5mL of distilled water and working standard respectively. The clear supernatants (0.5 mL) were transferred into another test tube. Freshly prepared combined colour reagent (0.25 mL) was added to the blank, working standard and each of the test samples. The resultant solutions were thoroughly mixed, stopped and incubated in water- bath at 56°C for 1 hour. The test tubes were then cooled in an ice-bath for about 5minutes. Ice cold 25% sulphuric acid (1 mL) was added slowly, drop by drop, to each test tube with mixing. The test-tube were allowed to stand at room temperature for 30 minutes, remixed and the absorbance then read against the blank at 500 nm. The plasma

ascorbic acid concentration for individual was calculated as follow:

(Absorbance sample / Absorbance standard) × 2.0 mg/dL = mg/dL ascorbic acid

3.2.2 Alpha-tocopherol assay (Vitamin E)

Analytic protocol: A volume of 0.4 ml of tissue homog enate was put into 8x75nm test tubes. An equal volume of purified absolute ethanol was added to the tube for protein precipitation. The contents were immediately mixed with a vortex mixer. Then 0.4 mL of xylene was added and the test tube were mixed for at least 30sec, and centrifuged for 5-10 min. at 3000 rpm. After centrifugation, the upper xylene layer which contains the extracted tocopherol is collected with a medicinal dropper (or by carefully decanting) and transferred to a small tube. When a great number of samples are handled, it is recommended to cover the tubes with parafilm to avoid evaporation. An exact volume of 0.2 mL, of serum-xylene extract was pipette into test tubes containing 0.1 mL. of BA. A volume of 0.1 mL. ferric chloride was added, followed by 0.1mL of orthophosphoric acid. The contents of the tube were mixed thoroughly using a vortex mixer after every addition of reagents. The order of reagent addition is critical. Absorbance was read in a spectrophotometer at 536 nm after setting the instrument to zero absorbance with a blank (prepared by using 0.2 mL. xylene instead of serum-xylene extract). A standard curve is constructed using the following procedure: 0.02 - 0.2 mL. of the diluted standard tocopherol solution (volume chosen so that there are 20µg tocopherol per millilitre) were measured into the analysis tubes, and the volume completed to 0.2 ml wherever necessary. These tubes were treated as are the 2 ml serum xylene extracts after centrifugation, as described above.

The standard curve was repeated (4 values) for each set determinations of unknown samples, as slight (<10%) variations in the slope of the standard curve were observed from day to day. The absorbance was read at 536nm using spectrophotometer.

***In vitro* anti-oxidant assays:**

Quantitative DPPH radical-scavenging assay: Scavenging activity on DPPH free radicals by the tissue homogenate was assessed according to the method reported by Gyamfi et al. [17] with slight modifications. Briefly, a 2.0 mL solution of the tissue homogenate at different concentrations diluted two-fold (2–1000 µg/mL) in methanol was mixed with 1.0 mL of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 mL) and 1.0 ml of methanol while the negative control was 1.0 mL of 0.3 mM DPPH solution plus 2.0 mL of methanol. L-ascorbic acid was used as the positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with a UV-visible spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated using the equation:

$$\% \, Inhibition = 100\,\% \times \left(\frac{A_0 - A_s}{A_0}\right)$$

Where;

A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample. The IC_{50} value represented the concentration of the sample extract that caused 50% inhibition of DPPH radical and was calculated by linear regression of plots, where the abscissa represented the concentration of tested sample and the ordinate the average percent of inhibitory activity from three replicates.

Superoxide radical ($O_2^{\cdot -}$)-scavenging assay: This assay was based on the capacity of the tissue homogenate to inhibit the photo-chemical reduction of nitro blue tetrazolium (NBT) [18] and the method used by Martinez et al. [19] to determine superoxide dismutase with slight modifications. Briefly, each 3.0 ml reaction mixture contained 0.05 M phosphate buffered saline (PBS) (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, NBT (75 µM) and 1.0 mL of test sample tissue homogenates (0–1000mg/mL). The tubes were kept in front of a fluorescent light (725 lumens, 34 watts) and absorbance was read at 560 nm after 20 min. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes containing reaction mixtures were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was estimated by comparing the absorbance of the control and those of the reaction mixture containing test sample as per

the equation:

$$\% \, Inhibition = 100 \, \% \times \left(\frac{A_0 - A_s}{A_0} \right)$$

Where, A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample.

3.3 Statistical Analysis

Data obtained from this study were analyzed using SPSS version 18.0 for windows and graphs plotted using Microsoft Excel 2010 for Windows 8.All the results are expressed as mean ± standard error of the mean (SEM) (n = 3).

4. RESULTS

4.1 Vitamin E Levels in Chicken, Beef and Fish Homogenates

As shown in Fig. 4 the level of vitamin E in the chicken homogenates (29.55 ± 4.84 µg\dL), and fish homogenates (34.47 ± 1.04 µg/dL) were significantly higher ($p<0.05$) compared to the levels in the Beef homogenates (19.16 ± 0.96 µg/dL).

4.2 Vitamin C Levels in Chicken, Beef and Fish Homogenates

In Fig. 5, the vitamin C levels in the Beef homogenates (51.12 ± 3.74) was significantly higher ($p<0.05$) compared to the chicken homogenates (28.60 ± 2.42 mg/dL) and fish homogenates (26.39 ± 1.63). However, there was no significant difference ($p>0.05$) in the vitamin C levels between the fish and chicken homogenates.

4.3 Free Radical Scavenging Activity

DPPH Radical Inhibition by Chicken, Beef and Fish Homogenates: As shown in Table 1, the fish and chicken homogenate showed significant dose-dependent DPPH radical inhibiting capacities, inhibiting 17.55± 3.71% and 16.61 ± 2.22% of DPPH at a concentration of 15 mg/mL compared to the Beef homogenate which inhibited 11.68 ± 1.78% at the same concentration. The IC_{50} of DPPH radical inhibitions were found to be 48.91± 3.19 and 47.83 ± 4.77 mg/mL for the chicken and fish homogenates as compared to the Beef homogenate at 72.04 ± 1.74 mg/mL relatively.

Superoxide ($O_2^{\cdot-}$) Anion Radical Inhibition by Chicken, Beef and Fish Homogenates: The homogenates inhibited the formation of reduced NBT in a dose-related manner. As shown in Table. 2, chicken homogenate showed the maximal O_2^- anion inhibitory activity of 65.26 ± 4.27% at the concentration of 15 mg/mL, compared to the fish homogenate with an inhibitory activity of 17.55 ± 3.71%, at 250 mg/mL. At all concentrations, superoxide anion radical inhibition was significantly higher ($p<0.05$) for the chicken and Beef homogenates compared to the fish homogenates. The IC_{50} of superoxide anion radical inhibition was found to be significantly lower ($p<0.05$) for the chicken and Beef extracts compared to the fish homogenate.

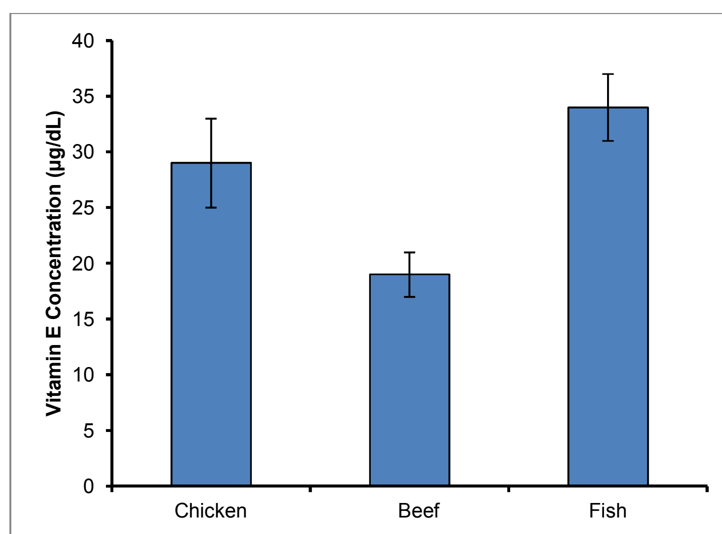

Fig. 4. Mean vitamin E levels in chicken, beef and fish homogenates

Table 1. DPPH radical scavenging activity of extracts

Concentration (mg/mL)	Inhibition (%)		
	Chicken homogenate	Beef homogenate	Fish homogenate
15.00	16.61 ± 2.22	11.68 ± 1.78	17.55 ± 3.71
7.50	12.06 ± 2.42	7.59 ± 1.14	14.92 ± 1.24
3.75	7.17 ± 1.09	5.50 ± 0.49	10.99 ± 0.77
1.87	6.64 ± 0.99	4.96 ± 0.17	8.92 ± 1.24
0.94	4.11 ± 0.24	2.44 ± 0.88	6.54 ± 0.75
0.47	2.33 ± 0.75	1.05 ± 0.49	4.72 ± 0.24
IC_{50}	48.91± 3.19	72.04 ± 1.74	47.83 ± 4.77

Table 2. Superoxide anion radical (O_2^-) inhibition by chicken, beef and fish homogenates

Concentration (mg/mL)	Inhibition (%)		
	Chicken homogenate	Beef homogenate	Fish homogenate
15.00	65.26 ± 4.27	56.16 ± 5.11	17.55 ± 3.71
7.50	55.50 ± 1.98	57.30 ± 1.84	14.92 ± 1.24
3.75	46.76 ± 2.03	35.50 ± 2.77	10.99 ± 0.77
1.87	36.08 ± 1.11	24.96 ± 1.86	8.92 ± 1.24
0.94	25.95 ± 2.27	18.95 ± 1.84	6.54 ± 0.75
0.47	19.37 ± 2.57	12.32 ± 1.40	4.72 ± 0.24
IC_{50}	7.57± 1.42	9.13 ± 1.10	48.14 ± 3.28

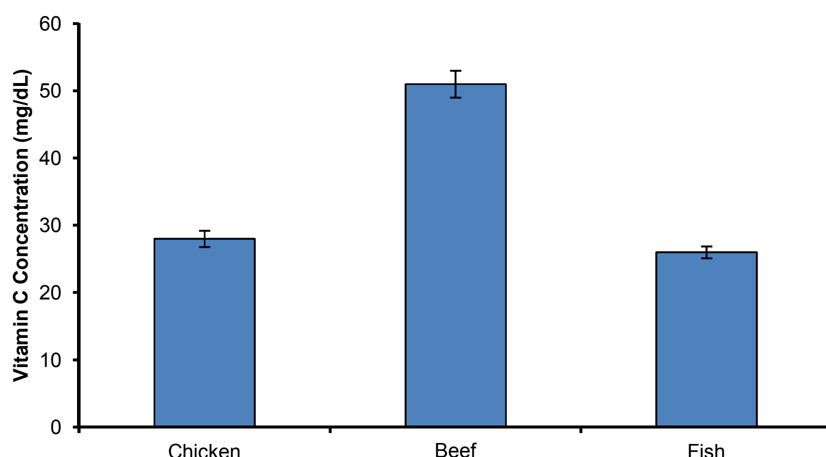

Fig. 5. Mean vitamin C levels in chicken, beef and fish homogenates

5. DISCUSSION

Animal tissues such as beef, chicken and fish are commonly used as food source. People take these Beefs basically for the purpose of adding proteins to their diet in other to make a balanced diet. These animal tissues have shown to be beneficial to health not only in protein production but also in free radical scavenging activity. Animal tissues have been in used since ancient time, since they are gotten from domestication or rearing them as food source or used in manufacture of certain product. In other to preserve and retain their nutritional values they are sometime subjected to various type/method of preserving foods such as drying, smoking, salting, refrigerating etc. Many foods in nature contain various types of reduced molecules such as phenolic compounds that contain electron-donor properties with antioxidant effect. In addition, they have the potential to contain several antioxidant enzymes and reduced coenzymes. They are rich in C and E vitamins that have high antioxidant effects and could include several elements with redox potential in their metabolism [20,21].

In the course of the this study I compared the antioxidant level of the animal tissues homogenate with buffer and xylene, by assessing the superoxide anion radicals($O_2^{\cdot-}$) , 2,2-diphenyl-1-picrylhydrazy(DPPH), Vitamin E and Vitamin C levels using appropriate assay system.

The antioxidant activity in fish, beef and chicken was investigated using Vitamin E assay. Statistically, fish showed a significantly ($p< 0.05$) high level of (34.47 ± 1.04 µg/dL) of vitamin E when compared with chicken homogenate (29.55 ± 4.84 µg\dL), and Beef homogenate (19.16 ± 0.96 µg/dL). This shows that fish have a higher level of vitamin E.

The antioxidant level of fish, Beef and chicken was investigated using Vitamin C assay. Statistically, Beef homogenate showed a significantly ($p< 0.05$) high level of (51.12 ± 3.74) of Vitamin C when compared to chicken homogenate (28.60 ± 2.42 mg/dL) and fish homogenates (26.39 ± 1.63). However, there was no significant difference ($p>0.05$) in the vitamin C levels between the fish and chicken homogenates.

DPPH radical is a stable free radical. It is associated with the use of DPPH method to search the in vitro antioxidant of compounds. Using animal tissue Beef , chicken, and fish homogenate, the fish and chicken homogenate showed significant dose-dependent DPPH radical inhibiting capacities, inhibiting 17.55 ± 3.71% and 16.61 ± 2.22% of DPPH at a concentration of 15 mg/ml compared to the Beef homogenate which inhibited 11.68 ± 1.78% at the same concentration. DPPH radical for the fish homogenate was significantly lower ($p< 0.05$) compared to chicken and Beef homogenate. Among the three homogenates, Beef homogenate was more efficient in inhibiting DPPH radical scavenger.

The antioxidant levels of animal tissues Beef, chicken and fish was investigated using superoxide anion radical, chicken homogenate showed the maximal O_2^- anion inhibitory activity of 65.26 ± 4.27% at the concentration of 15 mg/mL, compared to the fish homogenate with an inhibitory activity of 17.55 ± 3.71%, at 250 mg/mL. Although, at all concentrations superoxide anion radical inhibition was significantly higher ($p<0.05$) for the chicken and beef homogenates compared to the fish homogenates.

6. CONCLUSION

The results gotten from the various antioxidant assay using animal tissues, it can be said that animal tissues such as Beef, chicken and fish have significant antioxidant activities. The observed free radical action of the animal tissues could be of good relevance to food scientist. The consumption of animal tissues has beneficial effect and thus can be said that fish and Beef have higher antioxidant activity than chicken. Its encouraged to eat more of fish as it is capable of scavenging free radical and preventing oxidative stress and neuro degeneration not only production of protein in diet.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was granted to carry out the experimental work. The guideline for handling of laboratory animals during the research was followed. Also, work in conjunction with the technologists in the animal house and the laboratory.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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