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Evaluation of Antioxidant Activity of Methanol Extract of *Capsicum annum* (Red Bell Pepper), *Capsicum frutescens* (Chili Pepper), and *Capsicum annum* (Green Bell Pepper)

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Abstract: In This research, we focused on the evaluation of the antioxidant activity of three major categories of pepper grown in Nsukka, a small town located in Enugu State, in the Eastern part of Nigeria. The number of vitamins and organic compounds present in pepper may be affected by maturity, genotype, and processing. Also, the ascorbic acid content in peppers varies according to species, maturity, processing, and geographical location *Capsicum annum* (Red Bell Pepper), *Capsicum frutescens* (Chili Pepper), and *Capsicum annum* (Green Bell Pepper) species of pepper were obtained from the local market in Nsukka local government of Enugu State, Nigeria. These species of pepper were dried and ground individually. The different selected species of pepper (0.1 g) were treated with methanol, stirred for 45 minutes, and filtered before the antioxidant activity test was carried out using the DPPH assay technique, and Ascorbic acid as the standard control. Their absorbencies were obtained from the UV/V Spectrophotometer, and their% inhibition, mean and standard deviation values were also calculated according to the concentrations of the different sample solutions. *Capsicum annum* (Red Bell Pepper) showed the highest antioxidant activity, followed by *Capsicum frutescens* (Chili Pepper), while *Capsicum annum* (Green Bell Pepper) possessed the lowest antioxidant activity.

Keywords: Chili Pepper, Bell Pepper, Green Pepper, DPPH, Vitamin C and Antioxidant Activity, IC_{50}

1. Introduction

Pepper is one of the most important food spices originally domesticated in Mexico [1]. It is mainly used as a condiment and as a good food spicy used to color some raw foods before cooking. Pepper is grown in both wet and dry seasons, which are the only seasons in Nigeria. In the dry season, the demand for pepper is extremely high compared to the low harvest and supply of pepper [2]. This condition has caused the supply of pepper in Nigeria to attract so much profit due to its scarcity in every dry season [3]. Pepper is highly recognized for its medicinal purposes [4].

Pepper is an important source of Vitamin E, which protects the body against reactive oxygen, occasioned by oxidative stress. According to Mueller M. *et al*; antioxidants are groups of chemical species naturally found in our food that can prevent the oxidative stress of the physiological system [5]. Antioxidants are compounds that at minimal concentrations prevent the oxidation of a substrate [6]. Plants and animals have a complex body system of different types of antioxidants,

which includes: vitamin C and vitamin E, as well as enzymes, like catalase (CAT), superoxide dismutase (SOD), and various peroxidases [7]. The mechanisms of antioxidant activity of some plants have been reported. For example, the antioxidant activity of *Parinari kerstingii* leaf extract is due to its ability to neutralize the reactive oxygen species and prevent possible cellular damage and oxidative stress [8]. Oxidative stress is responsible for various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorders, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging [9]. Besides, there are reported antioxidants that cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet [10]. Antioxidants can also function as prooxidants when these are not present at the right place at the right concentration at the right time [11]. SOD is an enzyme that has been reported to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxynitrite. Therefore, by scavenging superoxide anions, it facilitates the activity of NO [12].

Catalase enzyme (CAT) is found in the blood and most of the living cells and decomposes H_2O_2 into water and oxygen. Catalase along with glucose peroxidase is also used commercially for the preservation of fruit juices, a cream consisting of egg yolk, and salad by removing the oxygen [12]. Non-enzymatic antioxidants are not found in the body naturally but need to be supplemented for proper metabolism [13]. Synthetic phenolic antioxidants are always substituted with alkyl groups to increase their solubility in fats and oils and reduce their toxicity [14].

Aside from vitamins A, E, and C, which are important antioxidants that protect unsaturated oil from being destroyed in the body by oxygen, and potent water-soluble antioxidants in humans [15], pepper also contains mixtures of antioxidants notably carotenoids, ascorbic acid, flavonoids, and polyphenols [16]. Pepper is increasingly becoming important as a food, medicinal, and industrial crop. Pepper contains organic compounds in fruits and vegetables that may be important in cancer chemoprevention [17].

The amount of vitamins A, C, and E content of pepper may be affected by maturity, genotype, and processing. Also, the ascorbic acid content in peppers varies according to species, maturity, processing, and geographical location [18]. Therefore, it is very pertinent to examine the antioxidant activity of Chili Peppers, Red Bell Peppers Green Bell Peppers, obtained from the Eastern part of Nigeria, which is the major objective of this work.

2. Materials and Method

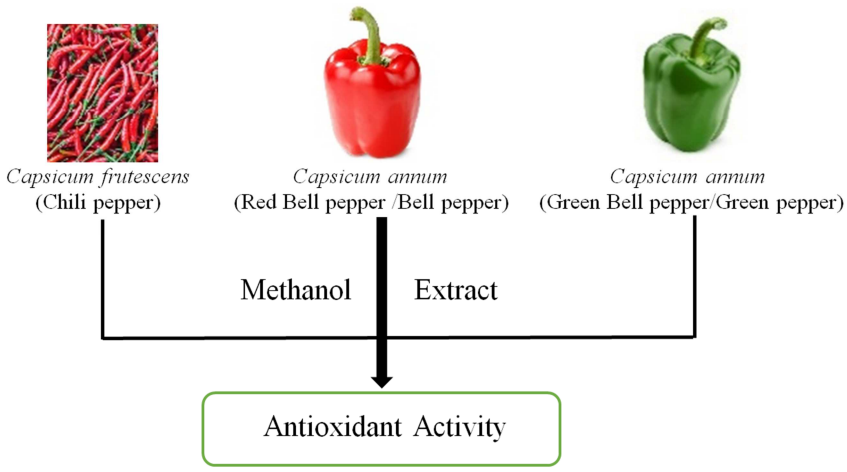

Figure 1. *The Flow Chart Methodology of Antioxidant Activity of Methanol Extract of the Three Categories of Pepper.*

2.1. Sample Preparation

The three different categories of pepper were collected from the local market at Nsukka community in Enugu state, Nigeria, and were identified and authenticated at the Department of Plant Science and Biotechnology as Red Bell pepper, Chili pepper, and Green Bell pepper. The peppers were ground to powder form and stored at 255.15 K in a domestic refrigerator until the extractions were performed.

2.2. Extraction and Drying Processes

The ground categories of pepper were dissolved in methanol and were allowed to stir overnight at room temperature. The organic layer was extracted using a separating funnel and concentrated in a rotary evaporator before they were laid differently under the sun for three weeks to dry properly.

2.3. Antioxidant Activity Test

The three different samples of pepper (0.1 g) were measured into various beakers, and 20 ml of methanol was added to the samples and stirred vigorously for 5 minutes each, they were filtered, and the filtrates were covered with aluminum foil and left for a few minutes. DPPH (0.0039g/L) was prepared and kept in a closed beaker due to its light sensitivity. The samples were measured and labeled in

different containers as it measured as follows: 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml. Then 0.1g of ascorbic acid (which is the standard solution used to carry out the experiment) was weighed, while 20 ml of methanol was added. Thereafter, the concentrations of the samples were increased, using a micropipette, to make each of the sample solutions have equal volume. There was an observable color change when DPPH was added to the sample solutions, and afterward, they were kept in a dark space. All the solutions with their different concentrations were now inserted into a UV/V spectrophotometer one after the other, to get their various absorbances. In the test for their absorbances, 20ml of methanol was used as the blank solution, while the spectrophotometer was set at 517 nm, which is where the maximum wavelength was obtained.

3. Results and Discussion

Table 1. *The concentrations and% inhibitions of Bell Pepper (BP), Chili pepper (CP), Green Pepper (GP) and Ascorbic acid.*

Dose mg/ml	Green Pepper (GP) % Inhibition	Chili Pepper (CP) % Inhibition	Bell Pepper (BP) % Inhibition	Ascorbic Acid % Inhibition
0.1	11.2903	43.6507	48.1415	44.3584
0.1	10.7526	39.4180	48.6773	44.0860
0.1	10.2151	42.8571	50.0000	45.6989
0.2	14.5161	47.6191	51.8519	53.2258
0.2	15.8602	50.0000	50.2646	52.4194
0.2	14.7895	50.5291	51.0582	51.8817
0.4	21.7742	51.5873	51.85185	62.0967
0.4	20.4301	51.0582	51.0582	61.2903
0.4	22.04301	50.5291	52.1164	60.4838
0.6	50.5376	52.6455	54.7619	70.9677
0.6	49.7312	53.1746	53.7037	72.0430
0.6	49.4624	53.9683	53.7037	71.7742
0.8	58.0645	53.4392	55.8201	77.9570
0.8	59.1398	54.2328	56.4392	77.3441
0.8	57.52688	55.0265	73.8095	76.3441
1.0	60.4839	70.6349	74.0740	86.0215
1.0	59.9462	72.7513	82.8042	84.9462
1.0	61.5591	71.6931	83.3333	84.1398

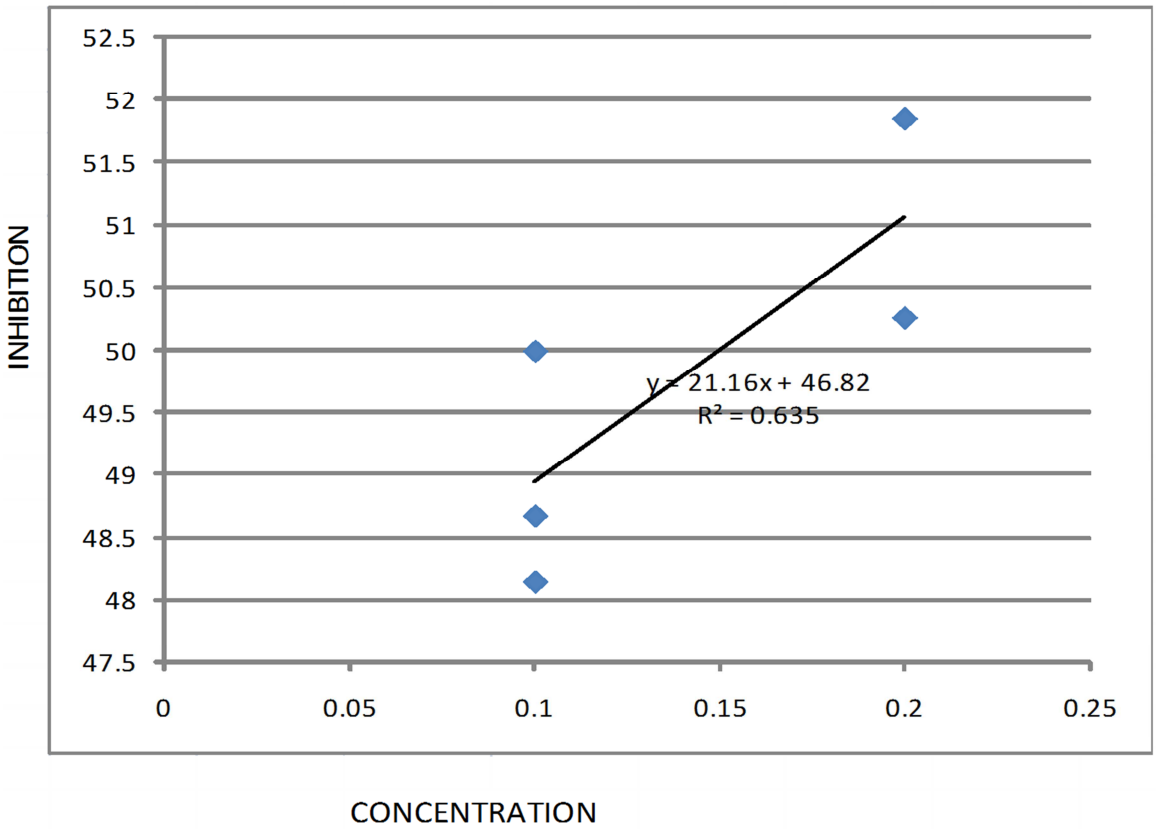

Figure 2. *Plot of % Inhibition Against Concentration For Bell Pepper (Bp).*

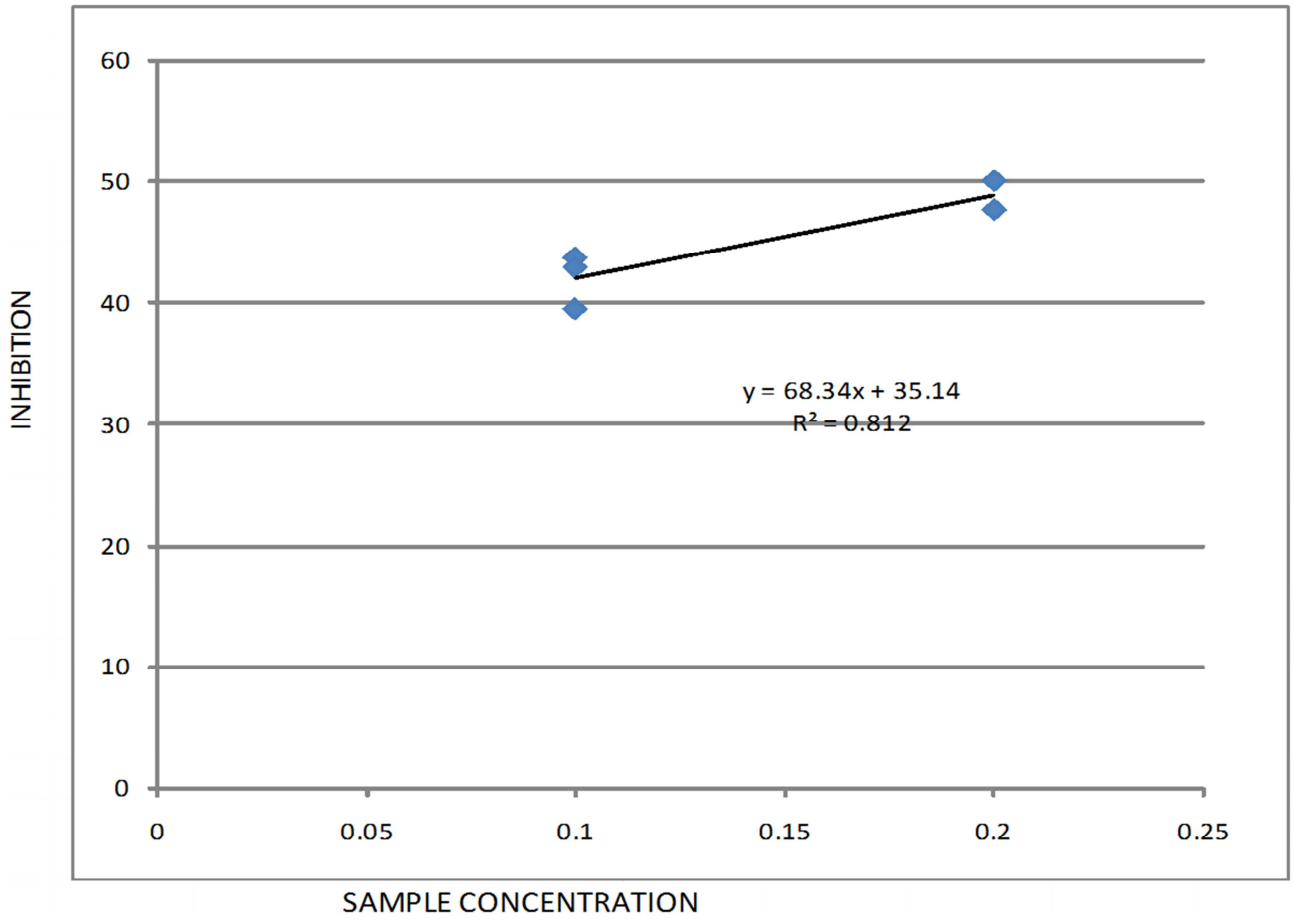

Figure 3. *Plot of % Inhibition Against Concentration For Chili Pepper (Cp).*

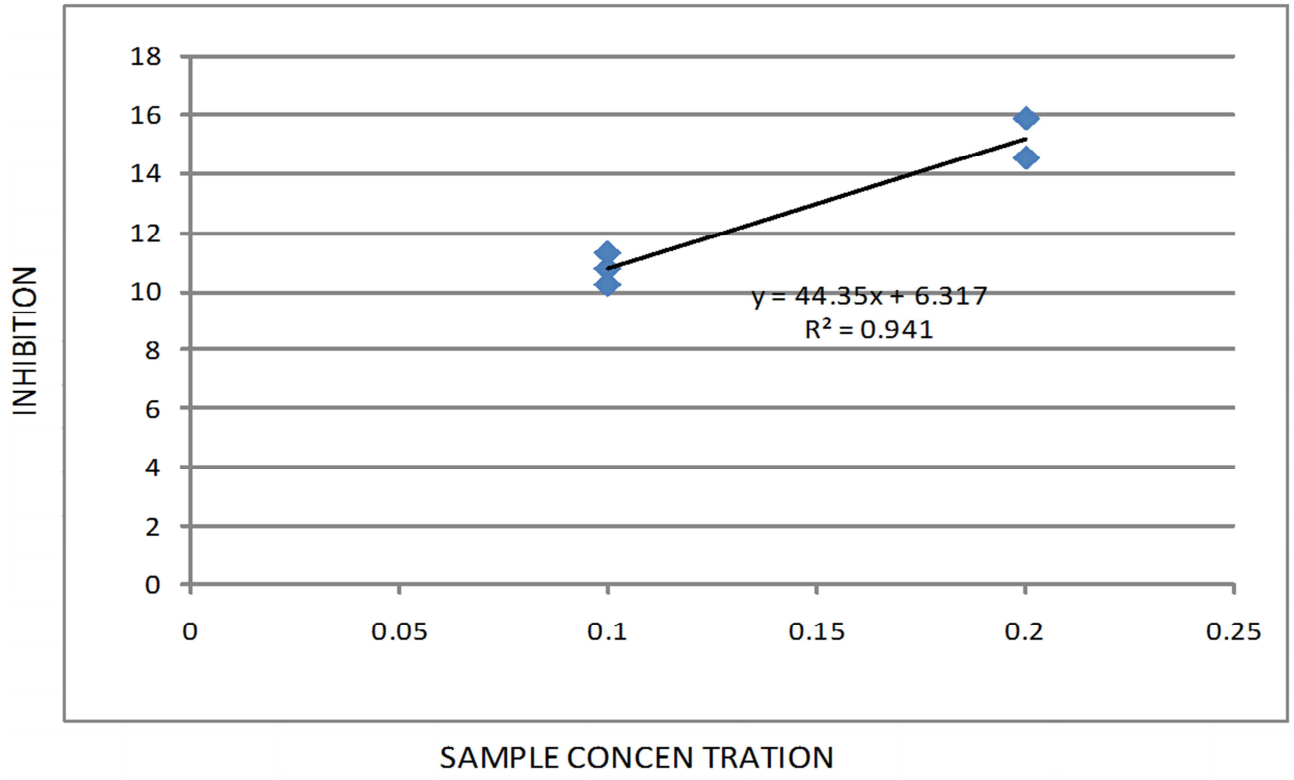

Figure 4. *Plot of % Inhibition Against Concentration For Green Pepper (Gp).*

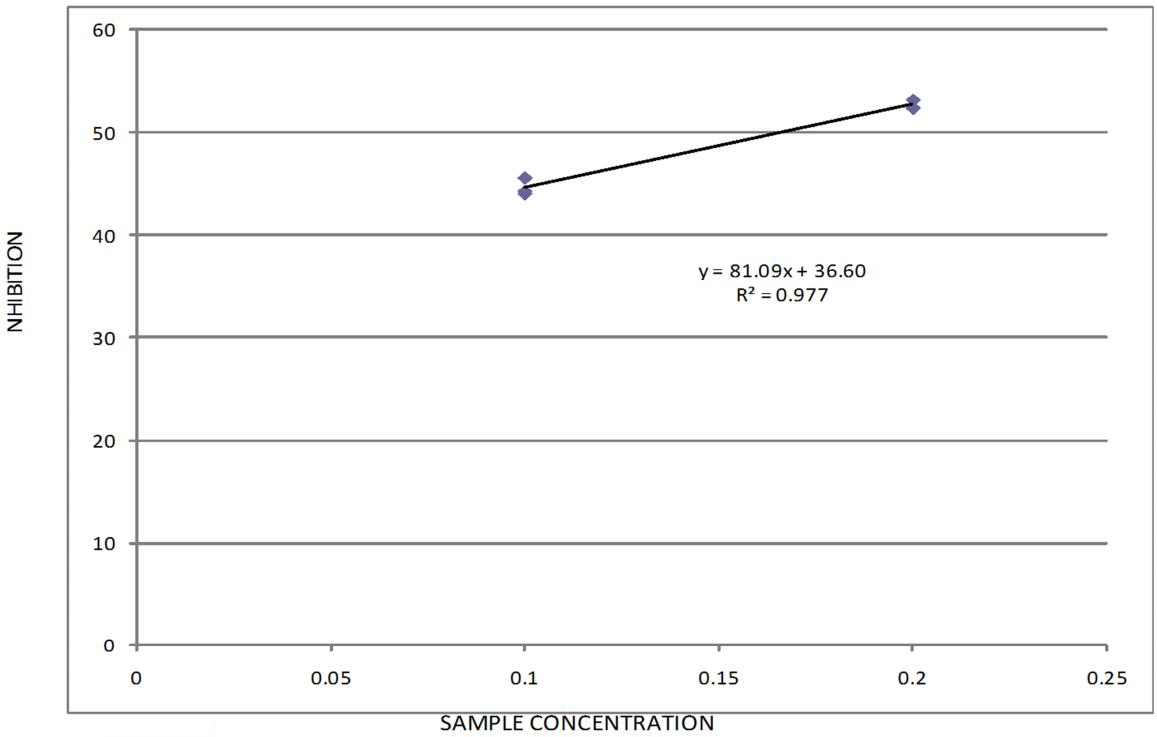

Figure 5. *Plot of % Inhibition Against Concentration For Standard (Ascorbic Acid).*

Table 2. *IC_{50} value for Bell Pepper (BP).*

CONCENTRATION OF BP (g/ml)	% INHIBITION (Mean & S. D)	IC_{50} (ppm)
0.1	48.942 ± 1.375	0.0558
0.2	51.658 ± 1.529	0.0558
0.4	51.675 ± 1.313	0.0558
0.6	54.057 ± 0.864	0.0558
0.8	61.993 ± 14.597	0.0558
1.0	80.070 ± 7. 354	0.0558

Table 3. *IC_{50} value for Chili Pepper (CP).*

CONCENTRATION OF CP (g/ml)	% INHIBITION (Mean & S. D)	IC_{50} (ppm)
0.1ml	41.975 ± 2.250	0.2174
0.2ml	49.383 ± 1.550	0.2174
0.4ml	51.058 ± 0.529	0.2174
0.6ml	53.263 ± 0.941	0.2174
0.8ml	54.233 ± 0.265	0.2174
1.0ml	71.693 ± 1.496	0.2174

Table 4. *IC_{50} value for Green Pepper (GP).*

CONCENTRATION OF CP (g/ml)	% INHIBITION (Mean & S. D)	IC_{50} (ppm)
0.1ml	10.753 ± 0.538	0.985
0.2ml	15.054 ± 0.711	0.985
0.4ml	21.417 ± 0.864	0.985
0.6ml	49.910 ± 0.559	0.985
0.8ml	58.244 ± 0.821	0.985
1.0ml	60.663 ± 0.581	0.985

Table 5. *IC_{50} value for Ascorbic Acid (Standard).*

CONCENTRATION OF CP (g/ml)	% INHIBITION (Mean & S. D)	IC_{50} (ppm)
0.1ml	44.713 ± 0.706	0.1652
0.2ml	52.509 ± 0.552	0.1652
0.4ml	61.290 ± 0.658	0.1652
0.6ml	71.595 ± 0.457	0.1652
0.8ml	77.151 ± 0.658	0.1652
1.0ml	85.036 ± 0.771	0.1652

The DPPH assay is used in this test because of its fastness, simplicity, inexpensive, and can be monitored over time (Siddartha, *et al.,* 2022). Table 1 shows the% inhibition of the three categories of pepper and that of the standard (ascorbic acid) at different dosages. Bell pepper gave the highest inhibition of 48% at the lowest concentration of 0.1 g/ml, more than the standard (ascorbic acid) that showed inhibition of 44% at the same concentration. Green pepper showed the least inhibition of 11% at the same concentration.

Tables 2-5 showed the IC_{50} values of Green Pepper, Bell Pepper, Chili Pepper, and the standard. IC_{50} is the concentration needed to inhibit a biological process by 50% (Julio, *et al.,* 2020). It is an important parameter employed to explain drug potency. The lower the IC_{50} value, the more potent the drug is, and vice versa. Among the three different categories of pepper chosen, Bell Pepper (*Capsicum annum*) has the highest antioxidant properties, with an IC_{50} value of 0.0558, followed by Chili Pepper (*Capsicum frutescens*), with an IC_{50} of 0.2174, while Green Pepper (*Capsicum*) has the lowest antioxidant activity, with an IC_{50} of 0.985. The ascorbic acid, which was used as the standard control has a $1C_{50}$ value of 0.1652 and therefore has an appreciable antioxidant activity, which is well known.

4. Conclusion

From the evaluations of the antioxidant activities of three different categories of pepper, it can be noted that pepper has tremendous antioxidant properties. In general, it is noted that low IC_{50} values denote high antioxidant activity, and the lowest IC_{50} values gotten in the test for antioxidant activity, were that of ascorbic acid and bell pepper, and in comparing both, it is observed that bell pepper has a lower IC_{50} value of 0.0558 than ascorbic acid, which has 0.1652, although it was used as the standard. Therefore, we can say that consuming Bell Peppers in whatever form possible, is more effective in protecting the body cells against free radicals that are formed, when the human body breaks down food (i.e. when they undergo oxidation) or when they are exposed to tobacco smoke and radiation. The increasing order of antioxidant activity is as follows:

Green pepper → Chili pepper → Ascorbic acid → Bell pepper

That is to say that bell peppers will inhibit or limit oxidation better, by restraining oxidative chain reactions than ascorbic acid, chili pepper, or green pepper.

Finally, pepper helps to protect the cells against free radicals, which cause heart disease, cancer, and other diseases. The antioxidants present in peppers generally help to protect the food and the body from oxidative damage induced by free radicals and reactive oxygen species. Peppers which can be used fresh, dried, fermented, or as an extract have both nutritional and nutraceutical importance. It contains an anticoagulant that helps prevent the blood clots that can cause heart attacks as well. It is also important to note that bell pepper itself is a good source of vitamin C.

5. Recommendation

Further studies are encouraged to isolate and characterize different fractions of bell peppers to determine the actual compound responsible for their antioxidant properties.

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Conflicts of Interest

The authors declare no conflicts of interest..

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