IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOL EXTRACT OF *CUCUMIS sativus*

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CERTIFICATION

This is to certify that this project work was carried out by CHITO GINIKACHUKWU JANE (LSC1705080), under the Supervision of PROFESSOR I.O. ONOAGBE and was submitted to the DEPARTMENT OF BIOCEMISTRY, UNIVERSITY OF BENIN in partial fulfillment of the requirements for the award of Bachelor of Science (B.SC) Degree in the department.

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DEDICATION

This project is dedicated to God Almighty who strengthened and sustained throughout the completion of this research work in good health.

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1.0. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Cucumber (*Cucumis sativus*), originated in India, belongs to family Cucurbitaceae, is most widely cultivated vegetable crop all over the world. Cucumber is the fourth most important vegetable crop after tomato, cabbage, and onion. Although its calorie and nutritional value is very low, it's a primary source of vitamins and minerals in the human diet (Mah, 1989). It's eaten in the unripe green form, the ripe yellow form normally becomes too bitter and sour. Antioxidants are substances that prevent and stabilize the damages caused by free radicals, it supplies electrons from antioxidants to damage cells.

Antioxidants turn free radicals into waste by-products, which are eliminated from the body. Consumption of anti-oxidant- enriched vegetables and fruits are known to reduce the risk of various diseases caused by free radicals. Such health benefits are mainly due to the presence of phytochemicals such as vitamin E, polyphenols and carotenoids.

1.2 Aim and Objectives

The aim of this study was to determine the antioxidant properties of aqueous and ethanol extracts of *Cucumis sativus*

The objectives were as follows:

1. Extraction of the whole plant fruit using distilled water or absolute ethanol
2. Determination of in vitro antioxidant activities of the aqueous and ethanol extracts.

1.3 Literature Review

1.3.1*Cucumis sativus*

Cucumis sativus (cucumber) the common cucurbitaceous summer vegetable known as cucumber (Cucumis sativus L.) is a prized annual crop grown for its immature fruits. Although cucumbers have very few calories, they are high in essential vitamins, minerals, and water. Numerous phytonutrients, such as alkaloids, tannins and flavonoids, are present in cucumber seeds.

There are three primary varieties of cucumber: slicing, pickling, and burpless/seedless. Cucumber is regarded as an annual plant.

1.3.2 Botanical Description

(Cucumis sativus) is a widely cultivated creeping vine plant in the Cucurbitaceae family that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiraling tendrils (Mariod *et al,* 2017).

As a warm-season crop, Cucumis sativus L. thrives in temperatures between 18 and 24 QC. It is not frost-resistant. All soil types, from sandy to loamy, are suitable for growing cucumbers. The best soil types for a higher yield include loam, slit loam, and clay loam. As a creeper, the cucumber plant has a tendency to climb or trail. The soft fruits can be eaten fresh or in salads with salt. Additionally, they are utilized as cooked veggies. Fruits are primarily utilized in salads and pickles. In some places the seeds are used in a variety of ways for human consumption. Greek historians also have recorded the various uses of cucumber. Cucumber was domesticated about 3000 years ago and is indigenous to India (Whitaker and Davis, 1962; Jeffrey, 1980; Robinson and Decker-Walters, 1997). Both in the summer and throughout the rainy season, cucumber is grown. Furthermore, it produces typically cylindrical fruits that are utilized as vegetables (Encyclopedia, 2019). The plant also has roots in a soilless media, which causes it to sprawl out across the ground instead of having a supporting framework. The vine has large leaves that shade the fruits. The root system is shallow and is primarily distributed in the 30 cm layer of cultivated ground. The stem is a vine with varying apical dominance. The epidermis of the stem has burrs, and the cross section of the stem is rhombic. On the stem, the axillae can branch, and the number of branches varies substantially between kinds. The cucumber's cotyledons are opposing and long elliptic, while the euphylla are alternate, simple, pentagonal palmate or cordate in shape, with 3–7 lobed blades. The axillary flower is unisexual, occasionally hermaphrodite, and unisexual. The flower is yellow, while the calyx is bristly green. Young fruit ranges in color from white to a pale green hue, and its shape is varied, including club-like, cylindrical, and

spherical. Every fruit contains 100–400 seeds. One thousand seeds weigh between 20 and 40 grams.

Cucumber is originated from South Asia, but now is grown in many continents, different kinds of cucumber are traded on the global market. Cucumber fruits consist of 90 to 95% water, in biological terms the cucumber is classified as a pepo, a type of botanical berry with a hard outer rind and no internal division. However, much like tomatoes and squashes, it is often perceived, prepared, and eaten as a vegetable. Cucumber contains abundant nutrients and has crunchy texture and unique flavor, so it is a typical vegetable used for a various types of dishes. It is also essential for salad and smoothie. Cucumber is rich in superior hydration and phytochemicals, which have diverse health benefits including weight loss, anti-inflammation, remedy for multiple diseases (Oboh *et al,*. 2017).

1.3.3 Taxonomical Classification of Cucumis sativus

Kingdom	Plantae
Subkingdom	Viridiplantae- green plants
Infrakingdom	Streptophyta – land plants
Super division	Embryophtya
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants
Class	Magnoliopsida
Superorder	Rosanae
Order	Cucurbitales
Family	Cucurbitaceae – gourds, squashes, citrouilles, gourdes
Genus	Cucumis
Species	Cucumis sativus – garden cucumber

1.3.4 Nutritional Composition of *Cucumis sativus*

English, Zucchini and Pranic healed cucumbers contain the highest and lowest in Holenarasipur and Dotted variety among the varieties (Urooj *et al.,* 2016).

Types of Nutrients found in *Cucumis sativus*

Vitamin K	19%
Molybdenum	12%
Vitamin B5	6%
Magnesium	3%
Manganese	3%
Potassium	3%
Biotin	3%
Copper	4%
Vitamin C	4%
Vitamin B1	3%

1.3.5 Different types of nutrients found in *Cucumis sativus*

Cucumis sativus consists of a variety of flavoids, including vicenin, diosmetin, quercetin, naringenin, and theaflavanoside. Other lignans found in Cucumis sativus include Pinoresinol, Lariciresinol, and Secoisolariciresinol, as well as triterpenes including Cucurbitacin A, B, C, and D.

1.3.6 Different types of cucumber

The cucumber on the basis of market are classified into two types: those that are grown to eat fresh are called slicing cucumbers and those consumed as a processed product (processing or pickling types). The quality attributes for slicing cucumber and pickling cucumber varies.

1.3.6.1 Slicing cucumber

The main varieties of slicers mature on vines with large leaves that provide shading (Dublin, 2016). Slicers grown commercially for the North American market are generally longer, smoother, more uniform in color, and have much tougher skin. Slicing cucumber are commonly called fresh market varieties.

1.3.6.2 Pickling cucumber

Pickling cucumber has smaller length/diameter ratios than slicing cucumber and usually has lighter coloured skin with pronounced wart at the immature stage.Any cucumber can be pickled, but commercial pickles are produced from cucumbers that have been deliberately bred for uniformity in length to diameter ratio and absence of cavities in the flesh. Pickler cucumbers, also known as cucumbers used for pickling, reach lengths of 7 to 10 cm (3 to 4 in) and widths of 2.5 cm (1 in). Compared to slicers, picklers tend to be shorter, thicker, less-regularly shaped, and have bumpy skin with tiny white or black-dotted spines. Color can vary from creamy yellow to pale or dark green.

1.3.6.3 Flowering and pollination

Pollination is required to produce marketable cucumbers in most open-field production systems. Pollination of cucumber plants impacts the yield, size, and weight of fruit in non-parthenocarpic varieties. The majority of cultivars of cucumber are seeded and demand pollination. Many honey beehives are transported to cucumber fields each year right before bloom for this reason. In addition to bees, bumblebees and other bee species can pollinate cucumbers. In parts of the world where cucumbers have been introduced, honey bees and stingless bees have been recorded as the main groups of pollinators, in the United States, 28 species of bees were recorded visiting cucumber flowers in Ohio (Smith *et al*,

2013).Cucumber production in greenhouses uses bumble bees and hand pollination to achieve marketable yields, but the use of stingless bees has also been proven effective for this production type (dos Santos, 2008).

Most cucumbers that require pollination are self-incompatible, thus requiring the pollen of another plant in order to form seeds and fruit (Nonnecke, 1989). Some cucumber varieties are parthenocarpic, which lowers the quality of the food they produce by having their blooms produce seedless fruit without pollination.

1.3.6.4 Health benefits of cucumber

Cucumber contains important compounds known to possess medicinal potency against hypertension, cancer, and cholesterol synthesis inhibition, microbial agents (Jony*, et al*, 2013). They impart health benefits beyond basic nutrition with antioxidants having potentials in reducing the risk of several deadly diseases in man (Okwesili, *et al* 2015). They are of nutritional value in preventing chronic diseases such as cancer, cardiovascular diseases and diabetes.

Cucumber is remarkably helpful for overall health; it could relieve thirst as it is rich in moisture and vital nutrients that are necessary for human body (Phuoc, 2019). It contains important electrolytes, cucumber can help prevent dehydration during hot months. Cucurbitacin B is a natural substance that is discovered profusely in cucumbers, and it exerts anti-cancer potential primarily through apoptosis-induction in diverse human cancer cells (Gao *et al*., 2014). Also it was found that cucurbitacin B encompasses potent chemo preventive activity against human prostate cancer (Gao *et al.,* 2014). Cucumber peel is a good source of dietary fiber that helps reduce constipation and offers some assurance upon colon cancers eliminating toxic aggregates from the abdomen. Cucumbers are contained with unique antioxidants in moderate ratios such as Beta-carotene and alpha-carotene, vitamin C,

vitamin-A, zeaxanthin and lutein. These compounds help acts protecting collectors against oxygen-derived free radicals. Oxidative stress and carbonyl stress play at critical functions in the progression of diabetes and its associated difficulties over developing free radical generation and weakening antioxidant defense systems (Bellamakondi *et al.,* 2017). They also have a low score on the glycemic index (GI), which means they provide important nutrients without adding carbohydrates that can increase blood glucose. Cucumber has protective impacts on diabetes developments and is recognized as a reliable food for lowering oxidative stress and carbonyl stress apparent in the diseases of diabetes (Heidari *et al*., 2016).

Also, cucumber juice is extremely good for hair, skin and nails. Skin generates free radicals due to repeated sun exposure, which leads to oxidative stresses and inflammatory responses in the dermal layer of the connective tissues ending aging and harm to cell membranes and biological molecules (Uthpala *et al*., 2020). Cucumber is a rich source of ascorbic acid and has the potential of anti-hyaluronidase and anti-elastase ability which justifies the use of cucumber as a possible anti-wrinkle agent (Nema *et al*., 2011).

Furthermore, Cucumbers have moderate diuretic potential, which is reasonably attributed to their free-water, potassium and low sodium content. This helps in checking weight gain and high blood pressure. They are rich in potassium, cucumber helps to lower blood pressure. They are rich in vitamin K which plays vital role in the bone mass developing activity (Abulude *et al*., 2007). Cucumber contains several antioxidants such as flavonoids, triterpenes and lignans that have anti-inflammatory properties. Higher oxidant activity in cucumber could be attributed to vitamin C contents, phenolic acids and their derivatives in higher concentration (Jony *et al.,* 2013). Cucumber is also used as an Alkaline Diet, Alkaline foods due to triterpenes which may regulate immune system related diseases. Presence of antioxidants and minerals counteracts acidic PH within the human body (Mahato et al., 1997).

Cucumber is used for medicine production, phytochemicals (potential chemo-preventive) are used for diarrhea, gonorrhea, diabetes, hypertension, detoxification, serum lipids regulation, anti-oxidant and analgesic ailments.

1.3.6.5 Beneficial Effects of *Cucumis sativus*

● Detoxification and Hydration

Cucumber's 95–96% water content aids in meeting the body's daily water needs for metabolism and helps to keep the body hydrated. In addition, cucumber has cooling and soothing properties..

●Reduce blood pressure

An electrolyte called potassium aids in controlling how much salt the kidneys retain. Magnesium, potassium, and dietary fiber are all present in sufficient amounts in cucumbers. These nutrients are proven to reduce blood pressure, which lowers the risk of heart disease.

●Hepatoprotective activity

Cucumber juice's ability to counteract oxidative stress caused by cumene hydroperoxide has been demonstrated through research. Cucumber extracts have antioxidants and radical scavenging property (Heidari *et al., 2012).*The extract help to form intracellular ROS.

●Effect on Cancer

Research has proven that the antioxidant properties of cucumber juice can mitigate the oxidative stress brought on by cumene hydroperoxide. Cucumber extract is rich in bioactive compounds and have anticancer activity with cell lines of (IC50) with MCF 715.6 ± 1.3 and HeLa 28.2 ± 1(Tuama *et al*., 2019).

●Effect on **SKIN**

Cucumbers enhances beautiful and beneficial to the skin. When cucumber juice is applied to the skin, it becomes supple and radiant. Cucumber's anti-inflammatory properties naturally lighten our skin and lessen tanning. In addition, it avoids creases.

●Effect in hair, nails and breath

Cucumbers include silica, which contributes to nail strength and keeps them from becoming brittle. Cucumber phytochemicals eliminate the bacterium that produces bad breath in our mouths..

1.3.6.6 Diseases of *C. sativus*

The most important diseases of *Cucumis sativus* are: cucumber green mottle virus(CGMV), wilt, black rot spot and nematode. Resistance to these pathogens occurs in a lot of wild species. There are so many pathogens and micro organisms that cause injuries to the leaves of the plant and other plant parts (Gill *et al*, 2011). Frequent diseases also include fusarium wilt of cucumber caused by *Fusarium oxysp,* Angular leaf spot which is caused by a bacterium *pseudomonas syringae ,*Anthracnose caused by the fungus *colletotrichum orbiculare,* Downy mildew caused by *pseudoperonospara cubensis (* a fungus-like organism).

1.4 ANTIOXIDANTS

Antioxidants are chemicals that stop the oxidation of other molecules from harming cells. An oxidizing substance gains electrons from one molecule through chemical events known as oxidation. Free radicals are known to be released during oxidation reactions. Antioxidants reacts with free radicals and terminates chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves. Both animals and plants

contain complex system of multiple types of antioxidants, such as vitamin E and vitamin C as well as superoxide dismustase and various peroxidases (Hamid *et al*… 2010).

There are various antioxidant assays namely

- Thiobarbituric acid Assay (TBARS)
- 2,2-Diphenyl-1-picrylhydrazyl (DPPH)
- Ferric Reducing/Antioxidant Power (FRAP)
- Total Alkaline Capacity (TAC)
- Nitric oxide scavenging capacity.

1.4.1● DPPH scavenging activity

The molecule 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of asubstrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.

1.4.2 ● Ferric reducing-antioxidant power (FRAP) assay

This technique assesses the antioxidants' capacity to lessen ferric iron. It is based on the reduction of 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) and ferric iron to the ferrous form at low pH. A diode-array spectrophotometer is used to measure the change in absorption at 593 nm in order to track this decline. The method created by Benzie and Strain can be used to conduct antioxidant assay (1999). FRAP values can be obtained by comparing the absorption change in the test mixture with those obtained from

increasing concentrations of Fe3+ and expressed as mM of Fe2+ equivalents per kg (solid food) or per L (beverages) of sample.

1.4.3 ● Reducing power method (RP)

Increasing the absorbance of the reaction mixtures serves as the foundation for this procedure. The action of the antioxidants is increased when the absorption increases. This technique measures the color of a complex formed by an antioxidant chemical, potassium ferricyanide, trichloroacetic acid, and ferric chloride at 700 nm. The reaction mixture's increased absorbance reveals the samples' reducing power (Jayaprakash et al., 2001)**.**

1.4.4 ● Nitric oxide scavenging activity

NO generated in biological tissues by specific nitric oxide synthases, metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction (*David, 1999, Ghafourifar and Cadenas, 2005, Marletta, 1989, Moncada et al., 1989; and Virginia et al., 2003*).

1.4.5 ● Thiobarbituric Acid Reactive substances Assay (TBARS)

In addition to a more focused assay like HPLC, thiobarbituric acid reactive substance is another way to find lipid peroxidation products in cells, tissues, and bodily fluids. (2018) (Shashank Kumar et al.). This test analyzes malondialdehyde (MDA), a split product of an unsaturated fatty acid endoperoxide produced by oxidation of lipid substrates. The MDA reacts with thiobarbituric acid (TBA) forming a pink chromogen, which is measured at 532-535 nm. In TBARS, the substrate becomes oxidized with the addition of a metal ion (copper,iron), a free radical generating compound (AAPH) followed by addition of TB. The extent of oxidation can be measured spectrophotometrically. The addition of any antioxidant moiety to the test solution inhibits the oxidation process, and the reduced chromogen formation indicates the antioxidant capacity.

CHAPTER TWO

2.0 MATERIALS AND METHODS

The research was conducted at the Department of Biochemistry, Faculty of Life Science, University of Benin, Edo state.

2.1 Reagents and Chemicals used

1diphenyl-2-picrylhydrazyl,potassium ferricyanide, sodium phosphate, ferricchloride, tripyridyltriazine, ammonium molybdate, sodium nitroprusside, Griess reagent, sulphuric acid, ascorbic acid, sodium dodecyl sulphate, trichloroacetic acid.

2.2 COLLECTION OF THE PLANT

Fresh mature cucumbers were purchased on January 2022 from a major market in Benin City, Edo State, Nigeria. The plant material was washed with distilled water and cleaned. The fruit was then cut into small pieces, air-dried and blended to obtain a powder.

2.3 Apparatus and equipment

- Digital weighing balance
- Conical flask
- Beakers
- Funnels
- Atomic absorption spectroscopy
- Gas chromatography
- Soxhlet apparatus
- Volumetric flask
- Fume cupboard
- Oven

- Measuring cylinder
- Syringe
- Micropitette
- Centrifuge
- Test tubes
- Clean plain containers
- Refrigerator
- Spectrophotometer
- Distilled water
- Water bath
- Spatula

2.4 Plant Extraction

Exactly 2g of fresh cucumber plant is weighed on the analytical weighing balance in three portions. After weighing, it is placed in the oven for three hours. After three hours it is brought out and weighed again and placed back into the oven for 30 minutes, after 30 minutes it is brought out and weighed on the analytical weighing balance .the weight is recorded. The ethanol extracts were rotary evaporated to concentrate them, then lyophilized to turn them into powder.

2.5. DPPH Assay

The free radical scavenging capacity of the plant extract against 1, 1-diphenyl-2-picrylhhydrazl (DPPH) free radical was determined by a slightly modified method of Brand-Williams et al., (1995). 0.3 ml of 0.5 ml DPPH solution in ethanol was added to 2ml of different extract concentrations ranging from 0.2 to 1.0 mg/mL. After 15 minutes of shaking and incubation in the dark at room temperature, the test tubes' absorbance at 517 nm was

measured. Each test was run three times. With quantities similar to those of the test materials, vitamin C, also known as ascorbic acid, was used as a control. As the test samples, a blank comprising 0.5 mL of 0.3 mM DPPH and 2 ml of ethanol was prepared. The radical scavenging activity was calculated as shown in Equation 1:

DPPH radical scavenging activity (%) $= A_0 - \underline{A_1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots$ *(1)*

Where A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard.

2.5.1 Ferric Reducing Anti-oxidant Property Assay (FRAP)

For the FRAP assay, a modified version of Benzie and Strain's technique was employed. The key principle is the sample's capacity to convert ferrous tripyridyltriazine (Fe (III)-TPTZ), which at low pH generates a bright blue color that can be read at 593 nm, from ferric tripyridyltriazine (Fe (II)TPTZ) complex. 1.5mL of freshly made FRAP solution, which is made up of 25mL of 300mM acetate buffer, 2.5mL of 10mM 2,4,6-tripyridylstriazine (TPTZ) in 40mM HCl, and 2.5mL of 20mM ferric chloride (FeCl3. 6H20) solution was combined with 1mL of extracts at various doses (0.2 - 1.0 mg/mL). For 30 minutes, the reaction mixtures were incubated at 37°C, and the absorbance was measured at 593nm. FeSO4 was utilized for calibration, and data were reported as mmol FeSO4 equivalents per gram of sample. Ascorbic acid served as the control.

2.5.2 Reducing Power Assay

The reducing power (RP) of extract was determined according to the method described by Lai *et al.,* (2001). 1mL of various extract concentrations (0.1-1.0 mg/mL) in water, 1mL of 0.2M phosphate buffer (pH 6.6), and 1mL of 1% potassium ferricyanide were combined. For 20 minutes, the mixture was incubated at 50°C. To stop the reaction, 2.5mL of 10%

trichloroacetic acid was then added to the mixture. After adding 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl3, the absorbance was measured at 700 nm. While ascorbic acid served as the control, higher absorbance levels showed more reducing power.

2.5.3 Total Antioxidant Capacity (TAC)

The TAC of the extracts was evaluated using the phosphomolybdenum method based on the procedure described by Prieto et al., (1999). The assay is based on the extracts' conversion of Mo (+6) to Mo (+5) and the phosphate Mo (+5) complex that results at an acidic pH. In a nutshell, 3mL of reagent solution was combined with 0.3 mL of graded quantities of extracts (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After cooling to room temperature, the reaction mixture's absorbance at 695 nm was measured using a spectrophotometer against a blank. The blank was made up of 0.3 mL of ethanol rather than extract. The TAC was expressed as milligram equivalents of ascorbic acid and calculated as shown in Equation

TAC (mg AAE/g extract) = C × V..... (2)

where c = concentration of ascorbic acid in mg/mL extrapolated from the standard calibration curve; V = volume of extract in mL; and m = weight of crude plant extract in grams

2.5.4 Nitric Oxide Radical Scavenging Capacity

The method described by Makhija et al., (2011) was used. Briefly, extract produced in phosphate buffer was combined with 1 mL of 10 mM sodium nitroprusside. 150 minutes were spent incubating the mixture at 25 °C. 1 mL of Griess' reagent was added to 1 mL of the incubated solution. At 546 nm, the absorbance was then measured.The % inhibition of nitric oxide radical was calculated as shown in Equation 3:

Nitric oxide scavenging activity (%) = Acontrol – Aextract x 100 ……(3)

2.5.5 Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric Acid Reactive Substances (TBARS) was estimated according to the method described by Ohkawa et al., (1979). In a test tube, 0.5 mL of 10% v/v egg yolk homogenate and 0.1 mL of extract were combined. 1 mL of distilled water was then added. Then, to cause lipid peroxidation, 50 L of FeSO4 (0.07 M) was added, and the mixture was incubated for 30 min. After that, 1.5 mL of 0.8% TBA in 1.1% sodium dodecyl sulphate (SDS) and 50 L of 20% TCA were added, and the mixture was vortexed. The resulting mixture was heated for 60 minutes at 95 oC. At 532 nm, the sample's absorbance was measured.

Inhibition of lipid peroxidation (%) was calculated as shown in Equation 4:

inhibition of lipid peroxidation (%) = $A_c - A_e$ x 100 ..... (4)

Acontrol

CHAPTER THREE

RESULTS

3.0 *In Vitro* Antioxidant Activities of Extracts of *C. sativus*

The DPPH radical scavenging activity, NO scavenging capacity and TBARS of the ethanol extract were significantly higher than those of aqueous extract ($p < 0.05$). However, reducing power, FRAP and total antioxidant capacity (TAC) of the aqueous extract were significantly higher than those of ethanol extract ($p < 0.05$). These results are shown in Tables 3.1 to 3.5 and Figure 3.1.

Table 3.1: DPPH Radical Scavenging Activity of Extracts of *C. sativus*

Concentration Of Extract (mg/mL)	**Inhibition (%)**		
	Aqueous	**Ethanol**	**Ascorbic Acid**
0.20	65.48 ± 1.35	91.63 ± 0.35	88.80 ± 3.72
0.40	46.66 ± 5.47	91.70 ± 0.36	88.57 ± 3.84
0.60	31.28 ± 2.26	91.94 ± 0.20	93.20 ± 0.18
0.80	20.83 ± 2.05	88.19 ± 1.84	90.58 ± 1.17
1.00	16.58 ± 1.50	89.22 ± 0.56	93.54 ± 1.33

Data are DPPH radical scavenging activity and are expressed as mean ± SEM (n = 3).

Table 3.2: NO Radical Scavenging Activity of Extracts of *C. sativus*

Concentration Of Extract (mg/mL)	NO Scavenged (%)		
	Aqueous	**Ethanol**	**Ascorbic Acid**
0.20	48.03 ± 0.00	87.90 ± 0.85	71.67 ± 0.50
0.40	43.53 ± 0.00	83.12 ± 1.51	-
0.60	57.13 ± 13.98	75.86 ± 2.38	75.42 ± 0.94
0.80	59.50 ± 0.00	67.29 ± 1.82	-
1.00	55.35 ± 0.94	55.82 ± 0.00	73.92 ± 1.73

Data are percentage NO scavenged *in vitro* and are expressed as mean ± SEM (n = 3).

Table 3.3: Effect of Extracts of *C. sativus* on TBARS

Concentration Of Extract (mg/mL)	Inhibition of Lipid Peroxidation (%)		
	Aqueous	**Ethanol**	**Ascorbic Acid**
0.10	22.34 ± 0.00	49.14 ± 10.65	43.47 ± 2.92
0.50	18.90 ± 7.56	43.30 ± 7.90	36.77 ± 1.03
1.00	16.67 ± 2.92	40.55 ± 0.00	40.43 ± 13.76

Data are percentage inhibition of lipid peroxidation and are expressed as mean ± SEM (n = 3).

Table 3.4: Reducing Power of Extracts of *C. sativus*

Concentration Of Extract (mg/mL)	**Absorbance at 700 nm**		
	Aqueous	**Ethanol**	**Ascorbic Acid**
0.20	0.605 ± 0.145	0.305 ± 0.005	0.823 ± 0.266
0.40	0.729 ± 0.196	0.355 ± 0.095	1.047 ± 0.391
0.60	0.873 ± 0.191	0.355 ± 0.085	1.210 ± 0.263
0.80	0.900 ± 0.105	0.340 ± 0.000	1.220 ± 0.010
1.00	1.287 ± 0.210	0.310 ± 0.000	1.115 ± 0.315

Data are reducing power of extracts of *C. sativus* and are expressed as mean ± SEM (n = 3).

Table 3.5: Ferric Reducing Antioxidant Potential (FRAP) of Extracts of *C. sativus*

Concentration Of Extract (mg/mL)	**Absorbance at 593 nm**		
	Aqueous	**Ethanol**	**Ascorbic Acid**
0.20	0.613 ± 0.032	0.157 ± 0.000	0.420 ± 0.000
0.40	1.057 ± 0.039	0.170 ± 0.030	0.610 ± 0.000
0.60	1.360 ± 0.029	0.195 ± 0.045	0.500 ± 0.000
0.80	1.530 ± 0.050	0.250 ± 0.020	0.560 ± 0.000
1.00	1.607 ± 0.037	0.320 ± 0.030	0.470 ± 0.000

Data are FRAP of extracts of *C. sativus* and are expressed as mean ± SEM (n = 3).

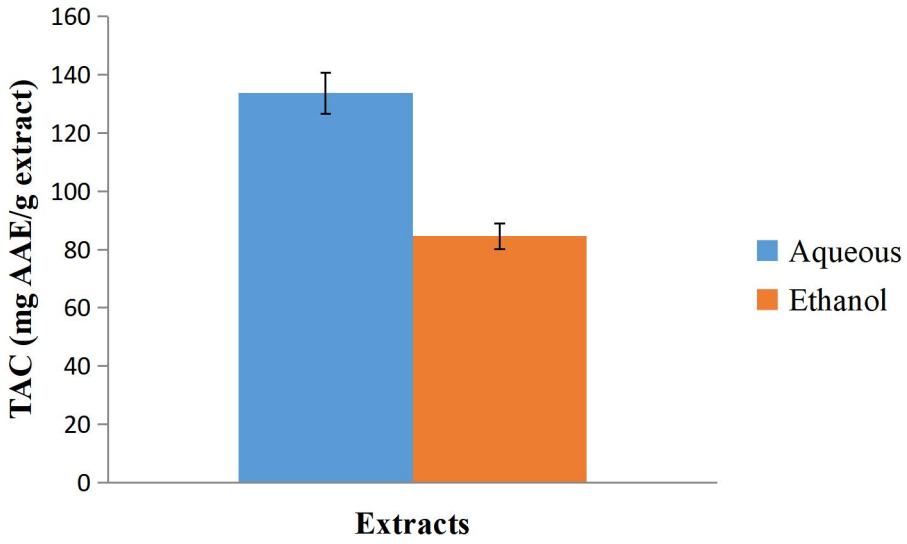

Figure 3.1: Total antioxidant capacity of extracts of *C. sativus*

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

Since an estimated 80% of the population in developing nations depends, at least in part, on medicinal plant materials for their primary healthcare, the quest for new natural chemicals with significant biological activity has been growing. The additional benefits of medicinal plant-based medications include their accessibility, effectiveness, and wide range of activity with a focus on prevention. These medicinal plants are usually known to exert their diverse health benefits through the numerous phytochemicals they contain (Jeyachandran *et al*., 2010; Jothy *et al.,* 2013; Manokaran *et al.,* 2008).

Living cells continuously produce free radicals, which are afterwards neutralized by antioxidant defenses. The primary line of defense against free radicals in animal and plant cells is provided by antioxidant enzymes. Antioxidant enzymes are expressed and controlled by a defense system when cells are subjected to oxidative stress as a defensive strategy against the harmful effects of free radicals. Before free radicals damage cellular components, antioxidant enzymes can stabilize or inactivate them (Teixeira et al., 1998). In order to stabilize the free radical, they either lower its energy or give up some of their own electrons for its use. Additionally, they can stop the oxidizing chain reaction to lessen the harm done by free radicals. More than sixty distinct health diseases, including aging, cancer, diabetes mellitus, Alzheimer's disease, strokes, heart attacks, and atherosclerosis, have reportedly been linked in a significant way by free radicals. The body's capacity to lower the danger of free radical-related health issues is made more apparent by lowering exposure to free radicals and increasing consumption of antioxidant enzyme rich foods or antioxidant enzyme supplements (Grazioli et al., 1998). Although synthetic free radical scavengers like butylhydroxyanisole

(BHA) and butylhydroxytoulene (BHT) exist, ongoing research into the potential antioxidant activities of natural plant parts is necessary due to worries about potential negative effects. By reducing the impact of free radicals, antioxidants help stop tissue damage. Scavengers are what they do. Nutritional antioxidants are essential in assisting in vivo antioxidant enzymes and molecules in the fight against free radicals. The DPPH radical can accept an electron or hydrogen ion to become a stable molecule (Du *et al*., 2009). Scavenging of DPPH radical is a widely used method for evaluating the free radical scavenging ability of plant or chemical materials (Lee *et al*., 2003).

The DPPH method is rapid, sensitive, and reproducible and requires simple conventional laboratory equipment for accessing antioxidant activity of samples (Du *et al*., 2009). Phenols and flavonoids represent phytochemicals whose relative abundance in plant extracts has been linked to antioxidant effect (Ayoola *et al*., 2008; Padmanabhan and Jangle, 2012). Reactive nitrogen species (RNS) are free radicals derived from the interaction of NO with oxygen or reactive oxygen species (ROS) (Tsai *et al*., 2007). Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide anion (Amaeze *et al*., 2011). Nitric oxide synthase (NOS), which has three isoforms: endothelium (eNOS), neuronal (nNOS), and inducible NOS, produces it (iNOS).Nitric oxide (NO) is generated from amino acid L-arginine by the enzymes in the vascular endothelial cells, certain neuronal cells, and phagocytes (Nagmoti *et al.*, 2011). In most circumstances, low NO concentrations are sufficient to affect the physiological actions of the radical. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities (Bhaskar and Balakrishnan, 2009). Chronic NO radical exposure has been linked to a number of carcinomas and inflammatory diseases include juvenile diabetes, multiple sclerosis, arthritis,

and ulcerative colitis. The toxicity of NO increases greatly when it reacts with the superoxide radical, forming the highly reactive peroxynitrite anion (ONOO−) (Amaeze *et al.,* 2011). Nitric oxide has been shown to be directly scavenged by flavonoids (Lakhanpal and Rai, 2007). Due to the reactivity of thiobarbituric acid (TBA) with several reactive substances in a biological sample, a more widely accepted terminology called TBARS is now commonly used (Sun *et al*., 2001). Thiobarbituric acid reactive substances (TBARS) is now considered as a standard marker for lipid peroxidation-induced oxidative stress (Tsai and Huang, 2015).

Antioxidant substances known as phenolic compounds function as free radical scavengers. The hydroxyl group (-OH), which is directly linked to an aromatic hydrocarbon (phenyl) ring, is thought to endow phenols with their antioxidant potential. Because of this, they readily give electrons to free radicals, which reduces their threat to live cells (Uyoh et al., 2013). Studies have revealed a direct relationship between total phenol content and antioxidant effect in different plants. High phenolic content-containing plant materials have high radical scavenging abilities (Ayoola *et al*., 2008; Ghasemi *et al*., 2009; Hegazy and Ibrahim, 2012). Antioxidant half-maximum inhibitory concentration (IC50) is the quantity needed to lower DPPH radical concentration by 50%. Since the relationship between it and antioxidant potential is inverse, a lower IC50 indicates a higher antioxidant potential (Chanda et al., 2011). According to the study's findings, the ethanol extract's DPPH radical scavenging activity, NO scavenging capacity, and TBARS were all noticeably higher than those of the aqueous extract. However, reducing power, FRAP and Compared to ethanol extract, TAC of the aqueous extract were much higher. These outcomes align with those of earlier research (Jayaprakasha *et al.,* 2001; Mamelona *et al.,* 2007; Osama *et al.,* 2009). In a study carried out by Osama *et al.,* it was reported that aqueous extracts from three sea cucumber species showed higher antioxidant effects than organic extracts, and it was concluded that most of the antioxidant compounds were hydrophilic (Osama *et al.,* 2009). In one study, it was suggested

that the presence of flavonoids and tannins in *C. sativus* extract was responsible for free radical scavenging and analgesic effects of the extract (Kumar *et al.,* 2010). Plants rich in polyphenols have been demonstrated to have high *in vitro* antioxidant activity (Abu *et al.,* 2020).

4.2 Conclusion

The results of this study indicate that extracts of *C. sativus* are effective in scavenging free radicals caused by oxidative stress, and the potency depends on the solvent used for extraction.

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APPENDICES

APPENDIX 1 (DPPH Activity)

Aqueous Extract	
R1	
Conc 1	
Conc 2	
0.705	64.10387
Conc 3	
1.152	41.3442
Conc 4	
1.413	28.05499
Conc 5	
1.63	17.00611
Conc 6	
1.697	13.5947
R2	
0.704	
0.833	
1.264	
1.543	
1.608	
R3	64.15479
0.625	57.58656
1.158	
1.372	35.64155
1.492	
1.61	21.43585
	18.12627

	68.17719 41.0387 30.14257 24.03259 18.02444
Ethanol Extract	
R1 0.165 0.177 0.155 0.199 0.196 R2 0.176 0.154 0.166 0.193 0.233 R3 0.152 0.158 0.154 0.304 0.206	 91.59878 90.98778 92.10794 89.86762 90.02037 91.0387 92.15886 91.54786 90.17312 88.13646 92.26069

	91.95519 92.15886 84.52138 89.5112
Ascorbic Acid	
R1 0.293 0.3 0.137 0.162 0.153 R2 0.147 0.149 0.13 0.208 0.101	 85.08147 84.72505 93.02444 91.75153 92.20978 \ 92.51527 92.41344 93.38086 89.40937 94.85743

ANOVA SUMMARY (Aqueous Extract)

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	65.4767	2.34129	1.35174	59.6606	71.2928	64.10	68.18
2	3	46.6567	9.46973	5.46735	23.1325	70.1808	41.04	57.59
3	3	31.2767	3.92059	2.26355	21.5374	41.0160	28.05	35.64
4	3	20.8267	3.54996	2.04957	12.0081	29.6453	17.01	24.03
5	3	16.5800	2.59000	1.49534	10.1461	23.0139	13.59	18.13
Total	15	36.1633	19.07808	4.92594	25.5982	46.7284	13.59	68.18

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	18.82000*	4.16075	.001	9.5493	28.0907
		3	34.20000*	4.16075	.000	24.9293	43.4707
		4	44.65000*	4.16075	.000	35.3793	53.9207
		5	48.89667*	4.16075	.000	39.6259	58.1674
	2	1	-18.82000*	4.16075	.001	-28.0907	-9.5493

		3	15.38000*	4.16075	.004	6.1093	24.6507
		4	25.83000*	4.16075	.000	16.5593	35.1007
		5	30.07667*	4.16075	.000	20.8059	39.3474
	3	1	-34.20000*	4.16075	.000	-43.4707	-24.9293
		2	-15.38000*	4.16075	.004	-24.6507	-6.1093
		4	10.45000*	4.16075	.031	1.1793	19.7207
		5	14.69667*	4.16075	.005	5.4259	23.9674
	4	1	-44.65000*	4.16075	.000	-53.9207	-35.3793
		2	-25.83000*	4.16075	.000	-35.1007	-16.5593
		3	-10.45000*	4.16075	.031	-19.7207	-1.1793
		5	4.24667	4.16075	.331	-5.0241	13.5174
	5	1	-48.89667*	4.16075	.000	-58.1674	-39.6259
		2	-30.07667*	4.16075	.000	-39.3474	-20.8059
		3	-14.69667*	4.16075	.005	-23.9674	-5.4259
		4	-4.24667	4.16075	.331	-13.5174	5.0241

*. The mean difference is significant at the 0.05 level.

Ethanol Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	91.6333	.61068	.35258	90.1163	93.1504	91.04	92.26
2	3	91.7033	.62581	.36131	90.1487	93.2579	90.99	92.16

3	3	91.9400	.33867	.19553	91.0987	92.7813	91.55	92.16
4	3	88.1867	3.17897	1.83538	80.2897	96.0837	84.52	90.17
5	3	89.2233	.97223	.56132	86.8082	91.6385	88.14	90.02
Total	15	90.5373	2.05639	.53096	89.3985	91.6761	84.52	92.26

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-.07000	1.26123	.957	-2.8802	2.7402
		3	-.30667	1.26123	.813	-3.1169	2.5035
		4	3.44667*	1.26123	.021	.6365	6.2569
		5	2.41000	1.26123	.085	-.4002	5.2202
	2	1	.07000	1.26123	.957	-2.7402	2.8802
		3	-.23667	1.26123	.855	-3.0469	2.5735
		4	3.51667*	1.26123	.019	.7065	6.3269
		5	2.48000	1.26123	.078	-.3302	5.2902
	3	1	.30667	1.26123	.813	-2.5035	3.1169
		2	.23667	1.26123	.855	-2.5735	3.0469
		4	3.75333*	1.26123	.014	.9431	6.5635
		5	2.71667	1.26123	.057	-.0935	5.5269
	4	1	-3.44667*	1.26123	.021	-6.2569	-.6365
		2	-3.51667*	1.26123	.019	-6.3269	-.7065
		3	-3.75333*	1.26123	.014	-6.5635	-.9431
		5	-1.03667	1.26123	.430	-3.8469	1.7735
	5	1	-2.41000	1.26123	.085	-5.2202	.4002
		2	-2.48000	1.26123	.078	-5.2902	.3302
		3	-2.71667	1.26123	.057	-5.5269	.0935
		4	1.03667	1.26123	.430	-1.7735	3.8469

*. The mean difference is significant at the 0.05 level.

Ascorbic Acid

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	88.8000	5.26087	3.72000	41.5329	136.0671	85.08	92.52
2	2	88.5700	5.43058	3.84000	39.7782	137.3618	84.73	92.41
3	2	93.2000	.25456	.18000	90.9129	95.4871	93.02	93.38
4	2	90.5800	1.65463	1.17000	75.7137	105.4463	89.41	91.75
5	2	93.5350	1.87383	1.32500	76.6993	110.3707	92.21	94.86
Total	10	90.9370	3.46117	1.09452	88.4610	93.4130	84.73	94.86

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	.23000	3.56320	.951	-8.9295	9.3895
		3	-4.40000	3.56320	.272	-13.5595	4.7595
		4	-1.78000	3.56320	.639	-10.9395	7.3795
		5	-4.73500	3.56320	.241	-13.8945	4.4245
	2	1	-.23000	3.56320	.951	-9.3895	8.9295
		3	-4.63000	3.56320	.250	-13.7895	4.5295

	4	-2.01000	3.56320	.597	-11.1695	7.1495
	5	-4.96500	3.56320	.222	-14.1245	4.1945
3	1	4.40000	3.56320	.272	-4.7595	13.5595
	2	4.63000	3.56320	.250	-4.5295	13.7895
	4	2.62000	3.56320	.495	-6.5395	11.7795
	5	-.33500	3.56320	.929	-9.4945	8.8245
4	1	1.78000	3.56320	.639	-7.3795	10.9395
	2	2.01000	3.56320	.597	-7.1495	11.1695
	3	-2.62000	3.56320	.495	-11.7795	6.5395
	5	-2.95500	3.56320	.445	-12.1145	6.2045
5	1	4.73500	3.56320	.241	-4.4245	13.8945
	2	4.96500	3.56320	.222	-4.1945	14.1245
	3	.33500	3.56320	.929	-8.8245	9.4945
	4	2.95500	3.56320	.445	-6.2045	12.1145

APPENDIX 2 (NO SCAVENGING ACTIVITY)

Aqueous Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	38.4600	13.53402	9.57000	-83.1384	160.0584	28.89	48.03
2	2	43.5300	.00000	.00000	43.5300	43.5300	43.53	43.53
3	2	57.1300	19.77071	13.98000	-120.5027	234.7627	43.15	71.11
4	2	59.5000	.00000	.00000	59.5000	59.5000	59.50	59.50
5	2	55.3500	1.32936	.94000	43.4062	67.2938	54.41	56.29
Total	10	50.7940	11.82745	3.74017	42.3332	59.2548	28.89	71.11

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-5.07000	10.73143	.657	-32.6560	22.5160
		3	-18.67000	10.73143	.142	-46.2560	8.9160
		4	-21.04000	10.73143	.107	-48.6260	6.5460

	5	-16.89000	10.73143	.176	-44.4760	10.6960
2	1	5.07000	10.73143	.657	-22.5160	32.6560
	3	-13.60000	10.73143	.261	-41.1860	13.9860
	4	-15.97000	10.73143	.197	-43.5560	11.6160
	5	-11.82000	10.73143	.321	-39.4060	15.7660
3	1	18.67000	10.73143	.142	-8.9160	46.2560
	2	13.60000	10.73143	.261	-13.9860	41.1860
	4	-2.37000	10.73143	.834	-29.9560	25.2160
	5	1.78000	10.73143	.875	-25.8060	29.3660
4	1	21.04000	10.73143	.107	-6.5460	48.6260
	2	15.97000	10.73143	.197	-11.6160	43.5560
	3	2.37000	10.73143	.834	-25.2160	29.9560
	5	4.15000	10.73143	.715	-23.4360	31.7360
5	1	16.89000	10.73143	.176	-10.6960	44.4760
	2	11.82000	10.73143	.321	-15.7660	39.4060
	3	-1.78000	10.73143	.875	-29.3660	25.8060
	4	-4.15000	10.73143	.715	-31.7360	23.4360

Ethanol Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	87.8950	1.19501	.84500	77.1583	98.6317	87.05	88.74
2	2	83.1150	2.12839	1.50500	63.9922	102.2378	81.61	84.62
3	3	75.8600	4.13068	2.38485	65.5988	86.1212	71.11	78.61
4	3	67.2900	3.15216	1.81990	59.4596	75.1204	64.54	70.73
5	2	55.8200	2.78600	1.97000	30.7888	80.8512	53.85	57.79
Total	12	73.5925	11.37984	3.28508	66.3621	80.8229	53.85	88.74

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	4.78000	3.11028	.168	-2.5747	12.1347
		3	12.03500*	2.83929	.004	5.3211	18.7489
		4	20.60500*	2.83929	.000	13.8911	27.3189
		5	32.07500*	3.11028	.000	24.7203	39.4297
	2	1	-4.78000	3.11028	.168	-12.1347	2.5747
		3	7.25500*	2.83929	.038	.5411	13.9689

	4	15.82500*	2.83929	.001	9.1111	22.5389
	5	27.29500*	3.11028	.000	19.9403	34.6497
3	1	-12.03500*	2.83929	.004	-18.7489	-5.3211
	2	-7.25500*	2.83929	.038	-13.9689	-.5411
	4	8.57000*	2.53954	.012	2.5649	14.5751
	5	20.04000*	2.83929	.000	13.3261	26.7539
4	1	-20.60500*	2.83929	.000	-27.3189	-13.8911
	2	-15.82500*	2.83929	.001	-22.5389	-9.1111
	3	-8.57000*	2.53954	.012	-14.5751	-2.5649
	5	11.47000*	2.83929	.005	4.7561	18.1839
5	1	-32.07500*	3.11028	.000	-39.4297	-24.7203
	2	-27.29500*	3.11028	.000	-34.6497	-19.9403
	3	-20.04000*	2.83929	.000	-26.7539	-13.3261
	4	-11.47000*	2.83929	.005	-18.1839	-4.7561

*. The mean difference is significant at the 0.05 level.

APPENDIX 3 (TBARS)

Aqueous Extract		*Inhibition of lipid peroxidation (%)*
R1		
Conc	1	49.48454
0.147		11.34021
Conc	2	-13.7457
0.258		
Conc	3	
0.331		22.33677
		26.46048
R2		19.58763
0.226		
0.214		
0.234		-68.3849
		-123.711
R3		-143.643
0.49		
0.651		
0.709		
Ethanol Extract		38.48797
R1		51.20275
0.179		26.11684
0.142		
0.215		59.79381
		35.39519
0.117		40.54983
0.188		
0.173		-47.7663
		-56.701
0.43		-157.388
0.456		

0.749	
Vitamin C	
R1	40.54983
0.173	35.73883
0.187	31.95876
0.198	46.39175
0.156	37.80069
0.181	67.35395
0.095	19.24399
0.235	28.52234
0.208	21.99313
0.227	

Aqueous Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	35.9100	19.19088	13.57000	-136.5132	208.3332	22.34	49.48
2	2	18.9000	10.69145	7.56000	-77.1589	114.9589	11.34	26.46
3	2	16.6700	4.12950	2.92000	-20.4321	53.7721	13.75	19.59
Total	6	23.8267	13.73058	5.60548	9.4173	38.2360	11.34	49.48

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	22.3400	.00000	.00000	22.3400	22.3400	22.34	22.34
2	2	18.9000	10.69145	7.56000	-77.1589	114.9589	11.34	26.46
3	2	16.6700	4.12950	2.92000	-20.4321	53.7721	13.75	19.59
Total	6	19.3033	5.72707	2.33807	13.2931	25.3135	11.34	26.46

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	3.44000	6.61715	.639	-17.6187	24.4987
		3	5.67000	6.61715	.455	-15.3887	26.7287
	2	1	-3.44000	6.61715	.639	-24.4987	17.6187
		3	2.23000	6.61715	.758	-18.8287	23.2887
	3	1	-5.67000	6.61715	.455	-26.7287	15.3887
		2	-2.23000	6.61715	.758	-23.2887	18.8287

Ethanol Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	49.1400	15.06137	10.65000	-86.1811	184.4611	38.49	59.79
2	2	43.3000	11.17229	7.90000	-57.0790	143.6790	35.40	51.20
3	2	40.5500	.00000	.00000	40.5500	40.5500	40.55	40.55
Total	6	44.3300	9.25890	3.77993	34.6134	54.0466	35.40	59.79

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	5.84000	10.82690	.627	-28.6160	40.2960
		3	8.59000	10.82690	.486	-25.8660	43.0460
	2	1	-5.84000	10.82690	.627	-40.2960	28.6160
		3	2.75000	10.82690	.816	-31.7060	37.2060
	3	1	-8.59000	10.82690	.486	-43.0460	25.8660
		2	-2.75000	10.82690	.816	-37.2060	31.7060

Ascorbic Acid

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	43.4700	4.12950	2.92000	6.3679	80.5721	40.55	46.39
2	2	36.7700	1.45664	1.03000	23.6826	49.8574	35.74	37.80
3	3	40.4333	23.83758	13.76264	-18.7825	99.6492	21.99	67.35
Total	7	40.2543	14.14622	5.34677	27.1712	53.3374	21.99	67.35

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	6.70000	16.99732	.714	-40.4921	53.8921
		3	3.03667	15.51636	.854	-40.0437	46.1170
	2	1	-6.70000	16.99732	.714	-53.8921	40.4921
		3	-3.66333	15.51636	.825	-46.7437	39.4170
	3	1	-3.03667	15.51636	.854	-46.1170	40.0437
		2	3.66333	15.51636	.825	-39.4170	46.7437

APPENDIX 4 (REDUCING POWER)

Aqueous Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	.6050	.20506	.14500	-1.2374	2.4474	.46	.75
2	3	.7293	.33918	.19582	-.1132	1.5719	.51	1.12
3	4	.8725	.38274	.19137	.2635	1.4815	.44	1.26
4	3	.9000	.18193	.10504	.4481	1.3519	.79	1.11
5	3	1.2867	.36364	.20995	.3833	2.1900	.87	1.54
Total	15	.8965	.35407	.09142	.7005	1.0926	.44	1.54

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-.12433	.29471	.682	-.7810	.5323
		3	-.26750	.27959	.361	-.8905	.3555
		4	-.29500	.29471	.340	-.9517	.3617
		5	-.68167*	.29471	.043	-1.3383	-.0250
	2	1	.12433	.29471	.682	-.5323	.7810
		3	-.14317	.24658	.574	-.6926	.4062

	4	-.17067	.26360	.532	-.7580	.4167
	5	-.55733	.26360	.061	-1.1447	.0300
3	1	.26750	.27959	.361	-.3555	.8905
	2	.14317	.24658	.574	-.4062	.6926
	4	-.02750	.24658	.913	-.5769	.5219
	5	-.41417	.24658	.124	-.9636	.1352
4	1	.29500	.29471	.340	-.3617	.9517
	2	.17067	.26360	.532	-.4167	.7580
	3	.02750	.24658	.913	-.5219	.5769
	5	-.38667	.26360	.173	-.9740	.2007
5	1	.68167*	.29471	.043	.0250	1.3383
	2	.55733	.26360	.061	-.0300	1.1447
	3	.41417	.24658	.124	-.1352	.9636
	4	.38667	.26360	.173	-.2007	.9740

*. The mean difference is significant at the 0.05 level.

Ethanol Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	.3050	.00707	.00500	.2415	.3685	.30	.31
2	2	.3550	.13435	.09500	-.8521	1.5621	.26	.45
3	2	.3550	.12021	.08500	-.7250	1.4350	.27	.44
4	2	.3400	.00000	.00000	.3400	.3400	.34	.34
5	2	.3100	.00000	.00000	.3100	.3100	.31	.31
Total	10	.3330	.06430	.02033	.2870	.3790	.26	.45

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-.05000	.08068	.563	-.2574	.1574
		3	-.05000	.08068	.563	-.2574	.1574
		4	-.03500	.08068	.683	-.2424	.1724
		5	-.00500	.08068	.953	-.2124	.2024
	2	1	.05000	.08068	.563	-.1574	.2574
		3	.00000	.08068	1.000	-.2074	.2074
		4	.01500	.08068	.860	-.1924	.2224

	5	.04500	.08068	.601	-.1624	.2524
3	1	.05000	.08068	.563	-.1574	.2574
	2	.00000	.08068	1.000	-.2074	.2074
	4	.01500	.08068	.860	-.1924	.2224
	5	.04500	.08068	.601	-.1624	.2524
4	1	.03500	.08068	.683	-.1724	.2424
	2	-.01500	.08068	.860	-.2224	.1924
	3	-.01500	.08068	.860	-.2224	.1924
	5	.03000	.08068	.725	-.1774	.2374
5	1	.00500	.08068	.953	-.2024	.2124
	2	-.04500	.08068	.601	-.2524	.1624
	3	-.04500	.08068	.601	-.2524	.1624
	4	-.03000	.08068	.725	-.2374	.1774

Ascorbic Acid

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	.8233	.46058	.26592	-.3208	1.9675	.43	1.33
2	3	1.0467	.67678	.39074	-.6346	2.7279	.40	1.75
3	3	1.2100	.45508	.26274	.0795	2.3405	.76	1.67
4	2	1.2200	.01414	.01000	1.0929	1.3471	1.21	1.23
5	2	1.1150	.44548	.31500	-2.8875	5.1175	.80	1.43
Total	13	1.0700	.43255	.11997	.8086	1.3314	.40	1.75

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-.22333	.40344	.595	-1.1537	.7070
		3	-.38667	.40344	.366	-1.3170	.5437
		4	-.39667	.45106	.405	-1.4368	.6435
		5	-.29167	.45106	.536	-1.3318	.7485
	2	1	.22333	.40344	.595	-.7070	1.1537
		3	-.16333	.40344	.696	-1.0937	.7670
		4	-.17333	.45106	.711	-1.2135	.8668

	5	-.06833	.45106	.883	-1.1085	.9718
3	1	.38667	.40344	.366	-.5437	1.3170
	2	.16333	.40344	.696	-.7670	1.0937
	4	-.01000	.45106	.983	-1.0501	1.0301
	5	.09500	.45106	.838	-.9451	1.1351
4	1	.39667	.45106	.405	-.6435	1.4368
	2	.17333	.45106	.711	-.8668	1.2135
	3	.01000	.45106	.983	-1.0301	1.0501
	5	.10500	.49411	.837	-1.0344	1.2444
5	1	.29167	.45106	.536	-.7485	1.3318
	2	.06833	.45106	.883	-.9718	1.1085
	3	-.09500	.45106	.838	-1.1351	.9451
	4	-.10500	.49411	.837	-1.2444	1.0344

APPENDIX 5 (FRAP)

Aqueous Extract

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	.6133	.05508	.03180	.4765	.7501	.55	.65
2	3	1.0567	.06807	.03930	.8876	1.2258	.98	1.11
3	3	1.3600	.05000	.02887	1.2358	1.4842	1.31	1.41
4	2	1.5300	.07071	.05000	.8947	2.1653	1.48	1.58
5	3	1.6067	.06429	.03712	1.4470	1.7664	1.56	1.68
Total	14	1.2121	.38395	.10262	.9905	1.4338	.55	1.68

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-.44333*	.04989	.000	-.5562	-.3305
		3	-.74667*	.04989	.000	-.8595	-.6338
		4	-.91667*	.05578	.000	-1.0428	-.7905
		5	-.99333*	.04989	.000	-1.1062	-.8805
	2	1	.44333*	.04989	.000	.3305	.5562
		3	-.30333*	.04989	.000	-.4162	-.1905

	4	-.47333*	.05578	.000	-.5995	-.3472
	5	-.55000*	.04989	.000	-.6629	-.4371
3	1	.74667*	.04989	.000	.6338	.8595
	2	.30333*	.04989	.000	.1905	.4162
	4	-.17000*	.05578	.014	-.2962	-.0438
	5	-.24667*	.04989	.001	-.3595	-.1338
4	1	.91667*	.05578	.000	.7905	1.0428
	2	.47333*	.05578	.000	.3472	.5995
	3	.17000*	.05578	.014	.0438	.2962
	5	-.07667	.05578	.203	-.2028	.0495
5	1	.99333*	.04989	.000	.8805	1.1062
	2	.55000*	.04989	.000	.4371	.6629
	3	.24667*	.04989	.001	.1338	.3595
	4	.07667	.05578	.203	-.0495	.2028

*. The mean difference is significant at the 0.05 level.

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	2	.1570	.00000	.00000	.1570	.1570	.16	.16
2	2	.1700	.04243	.03000	-.2112	.5512	.14	.20
3	2	.1950	.06364	.04500	-.3768	.7668	.15	.24
4	2	.2500	.02828	.02000	-.0041	.5041	.23	.27
5	2	.3200	.04243	.03000	-.0612	.7012	.29	.35
Total	10	.2184	.07025	.02221	.1681	.2687	.14	.35

Ethanol Extract

Multiple Comparisons

Dependent Variable:VAR00002

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-.01300	.04111	.765	-.1187	.0927
		3	-.03800	.04111	.398	-.1437	.0677
		4	-.09300	.04111	.073	-.1987	.0127
		5	-.16300*	.04111	.011	-.2687	-.0573
	2	1	.01300	.04111	.765	-.0927	.1187
		3	-.02500	.04111	.570	-.1307	.0807

	4	-.08000	.04111	.109	-.1857	.0257
	5	-.15000*	.04111	.015	-.2557	-.0443
3	1	.03800	.04111	.398	-.0677	.1437
	2	.02500	.04111	.570	-.0807	.1307
	4	-.05500	.04111	.239	-.1607	.0507
	5	-.12500*	.04111	.029	-.2307	-.0193
4	1	.09300	.04111	.073	-.0127	.1987
	2	.08000	.04111	.109	-.0257	.1857
	3	.05500	.04111	.239	-.0507	.1607
	5	-.07000	.04111	.149	-.1757	.0357
5	1	.16300*	.04111	.011	.0573	.2687
	2	.15000*	.04111	.015	.0443	.2557
	3	.12500*	.04111	.029	.0193	.2307
	4	.07000	.04111	.149	-.0357	.1757

*. The mean difference is significant at the 0.05 level.

APPENDIX 6 (TAC)

TAC (mgAAE/g extract)		**Absorbance at 695 nm**	
Aqueous	**Ethanol**	**Aqueous**	**Ethanol**
119.50	88.98	0.220	0.158
139.20	80.11	0.260	0.140
142.15	79.13	0.266	0.138
133.62 ± 7.11	**84.55 ± 4.44**		