Global Journal of Pure and Applied Chemistry Research
Vol.10, No.2, pp.28-41, 2022
Print ISSN: ISSN 2055-0073(Print),
Online ISSN: ISSN 2055-0081(Online)

Extraction of Phenolic Contents from *Cucurbita Pepo*

[*1]B. N., Abdulsalam, [2]S. M., Salisu, [*3]M., Salihu, [3]U. Abdulmumin
[*1]Department of Chemistry, Sa-adatu Rimi College of Education Kumbotso Kano, Nigeria
[2]Department of Pure and Industrial Chemistry, Bayero University Kano, Nigeria
[3]Department of Chemistry, Shehu Shagari College of Education, Sokoto, Nigeria
Corresponding Author Email: mustaphasalihu6773@gmail.com

Citation: Abdulsalam B.N., Salisu S.M., Salihu M., Abdulmumin U. (2022) Extraction of Phenolic Contents from Cucurbita Pepo, Global Journal of Pure and Applied Chemistry Research, Vol.10, No.2, pp.28-41

ABSTRACT: *Cucurbita p. is a commonly grown vegetable plant which is widely available in Southern African and other African states inculding Nigeria. However, Cucurbita belong to cucurbitaceae family generally known as pumpkin consumed as leafy, vegetable, fruits, flowers and seeds, which can then be simply boiled and eaten. Cucurbita p. contains as many important substances as possible such include calcium, iron, vitamins, oil, and protein etc and similarly provides natural antioxidants such as vitamin A, vitamin C, vitamin E, beta carotene which slow the process of aging by preventing free radicals from oxidizing sensitive biological molecules. Folin ciocalteu reagent was used to determine the total phenolic content present in the Cucurbita p. samples. The result indicated that, the total phenolic content in Cucurbita pepo fraction with petroleum ether is 17.1mg/g of garlic acid and methanol extract with 12.6mg/g showed high phenols while ethanol fraction was the lowest phenolic content with 8.0mg/g. The analysis using HPLC chromatogram shows presence of compounds at 254nm and at different retention times. Therefore, Cucurbita p. is a source of natural antioxidant due to the antioxidant activity determined.*

KEYWORDS: Cucurbita plant sample, antioxidant, Phenolic contents, Lowry solutions, Gallic acid solution.

INTRODUCTION

Cucurbita p. is a grown plant in Southern Africa and other African states including Nigeria. *Cucurbita p.* belong to the *cucurbitaceae* family, they are consumed as leafy vegetable, fruits, flowers and seeds, and they can be simply boiled and eaten. They are generally known as "pumpkin" and it is the most important plant that supply humans with edible products (Smith, 1997). *Cucurbita pepo* contains calcium, iron, vitamins, oil, and protein. *Cucurbita pepo* leaves is the most important vegetable in Moshna-land west province, it is consumed 3.9 times a week during rainy season in Moshna-land east province of Zimbabwe (Dersluijer *et al*, 1997). It produces a lot of nutrients required by humans. *Cucurbita* seed is a popular snack in several countries among of which is Greece. They are consumed it either as a raw or roasted and used in cooking and baking as an ingredient of bread, cereal, salad and cakes. Moreover, *cucurbita* seed

oil gain wide acceptance not only as edible oil but as nutraceutical. It has many health benefits and is a rich natural source of proteins, (Phillips, Ruggio, et al.,2005). In addition, *cucurbita* oils has been found to alleviate diabetes by promoting hypoglycemic activity (caili *et al.,* 2006). *Cucurbita* is an excellent fruit that has essential constituents required for good health of humans (Shrivastava and Roy, 2013).

Plate 1: *Cucurbita pepo* plant

Nutritional Values of *Cucurbita pepo*.

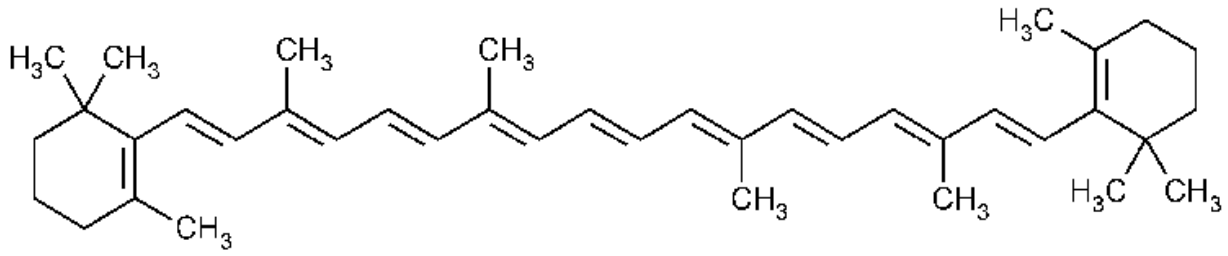
Cucurbita is widely grown vegetable that is incredibly rich in vital antioxidants and vitamins, while humble backyard ground variety is less in calories but contains many natural polyphenolic, flavaniodal compounds such as lutein, zea-xanthin and beta carotenes. Carotene serves as vitamin A source inside the body (Yadav *et al.,* 2010).

Ethnomedicinal Uses

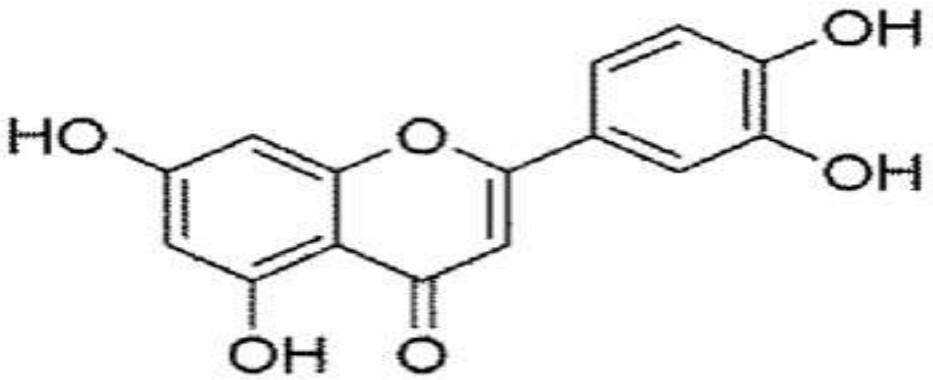
Cucurbitaceae family is commonly using as medicinal plants. These varieties of plants are used to heal burns, and abrasions, and are also used in respiratory disease, skin disorders, diuretics, essence and anti-inflammatory (Gilani, *et al*, 2005, Mukherjee and Wahile, 2006). The seeds are great sources of protein, magnesium, calcium and potassium. They are also used to push out the intestinal warms and tapeworms. *Cucurbita pepo* helps to eliminate pinworms from human body. Herbal medicine has a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness, they play major role in reducing the risks of heart diseases, diabetes, and some types of cancer (Aiyegoro, 2010).

Antioxidants

Antioxidant is a chemical compound that removes a chemical called free radical. Free radicals are atoms or molecules which contain unpaired electron. Electron has a very strong tendency to exist in a paired rather than unpaired state. This occurs naturally during the process of metabolism. The secondary metabolites that are water soluble and which possess antioxidants properties are; Phenolic acids, poly phenols, and flavaniods. They are natural phenols which protect plants from oxidative damage. They play major role in reducing the risks of heart diseases, diabetes, and some types of cancer (Moon, Shibamato, 2009). Free radicals are useful because they help important reactions in our bodies to take place. They can be manufactured as pharmaceuticals, plastics or other innovative materials. *Cucurbita pepo* provides natural antioxidants such as vitamin A, vitamin C, vitamin E, beta carotene which slow the process of aging by preventing free radicals from oxidizing sensitive biological molecules. The antioxidants found in many foods are frequently cited as the basis of claims for the benefits of a high intake of certain foods (e.g some vegetables and fruits) in the diet (Omenn *et al*.,1996).



Beta Carotene



Luteolin

Figure 1: Some antioxidant compounds isolated from plants

The leafy vegetable part produced by *cucurbita pepo* is one of the most palatable food ever known in the south west Nigeria. Vegetables constitute an important component in human diet, especially in developing countries, supplying essential mineral and vitamins that may not be obtained from staples. They produce more nutrients per unit land area than staples such as rice (AVRDC, 1990).

The health effects of fruits and vegetables have been attributed to the relatively high antioxidants they contain. (Ames *et al,* 1993, Evans and Miller, 1995)**.** Antioxidants are naturally present in fruit and vegetables, they are micro nutrients that have the ability to neutralize free radicals (Cadenzas and Packer, 1996; Nicoli et al., 1999). Free radicals have been implicated as the causative agent of several major human ailments, including cancer, cardiovascular diseases, and diabetes (Sies, 1996, Yoshikawa *et al.,* 2000, Devasagayam *et al.,* 2004).

Aims and Objectives

The research aimed to study the antioxidant capability of the extracts from *cucurbita pepo* the plant in comparison with black tea by using DPPH scavenging activity and total Phenolic content assays. Furthermore, it also aimed to identify the HPLC profiles of the compound present in *cucurbita pepo.*

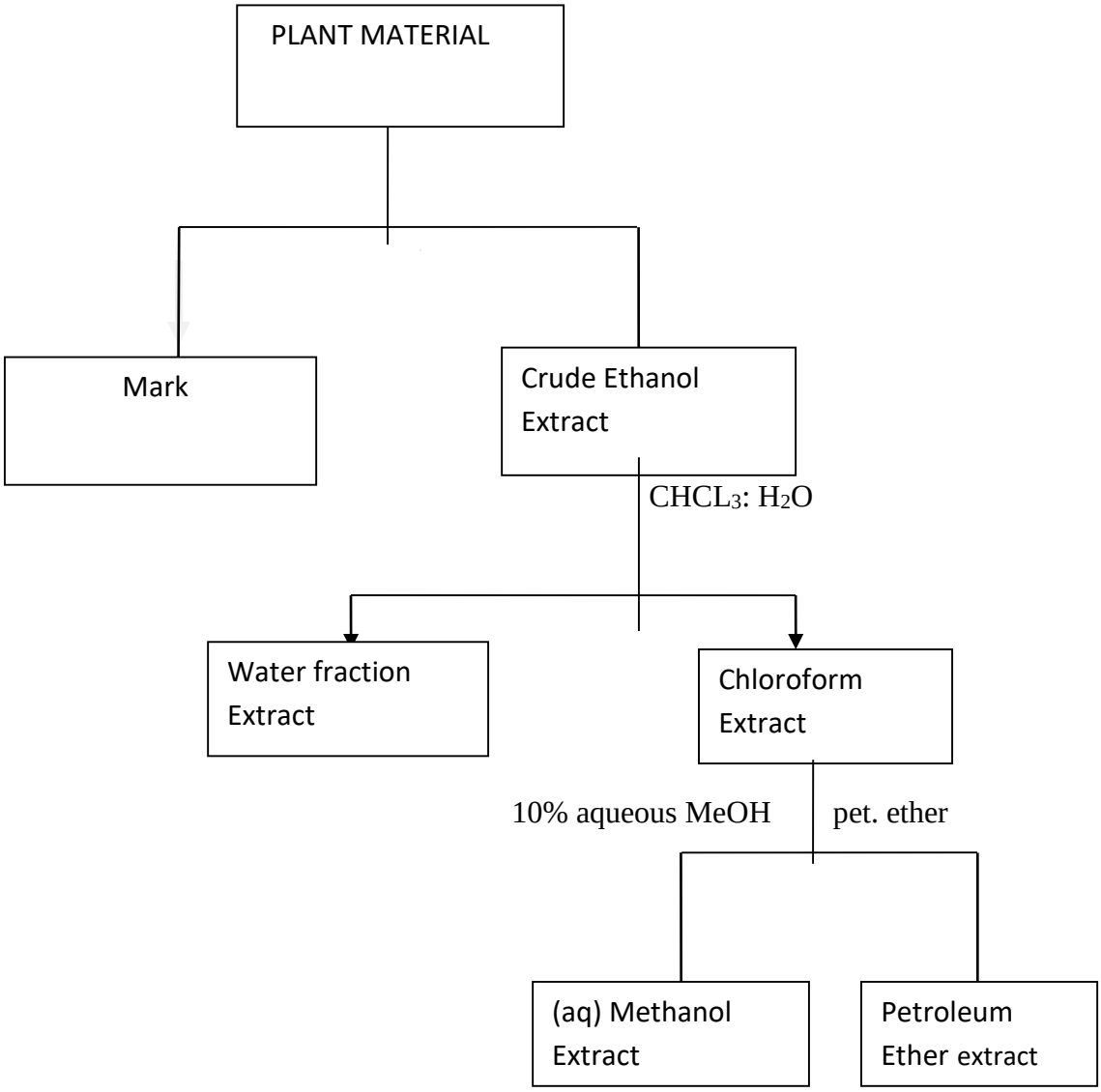
MATERIALS AND METHODS

Materials, Apparatus and Equipments

Weighing balance, HPLC machine, UV-lamp, IR-Spectrophotometer and UV-visible spectrophotometer were used from instruments laboratory. Solvents of analytical grade i.e. Ethanol, chloroform, methanol, pet Ether, were collected from department store or purchased from Sigma-Aldrich. Distilled water was collected from analytical laboratory. DPPH, Gallic acid, Folin-ciocalteu reagent, sample bottles, vials, and powdered leaves of *Cucurbita pepo* plant were obtained from Dr. Ahmad A Yakasai (supervisor).

Extraction

100g of the powdered plant was percolated for 7days in ethanol with continuous shaking. it was filtered and concentrated using rotary evaporator and allowed to dry under fan for three days. the dried sample was weighed and labeled as crude extract. Further extraction of the crude extract was achieved using separating funnel by using solvents of various polarities: (chloroform/water 1:1) and (pet ether/aqueous methanol 1:1). The extracts collected were concentrated, dried, weighed and labeled as F_5 and F_4.



Scheme 1: A chart of extraction processes

DPPH Assay

2mg of each of the extracts were weighed in a vial and 2ml of methanol was added, which resulted to a concentration of 10mg/ml (10,000µg/ml). This solution was labeled as stock solution. Serial dilution was employed for the preparation of the test solutions (1000-10µg/ml), using methanol. 0.1ml of the test sample was a liquated in a 96 well plate and 0.2ml of the prepared DPPH solution was added in duplicate. The mixture was incubated for 30mins and the absorbance was taken at 517nm using "micro plate reader".

$$Scavenging\ activity\ (\%) = \frac{[Abs\ S - Abs\ B]}{Abs\ C} x100$$

Where Abs S is the absorbance of sample, Abs B absorbance of blank and Abs C is absorbance of control.

Total Phenolic Content Assay

Preparation of Gallic acid solution.
10mg of pure garlic acid powder was weighed and dissolved in 1ml of methanol. This stock solution was used to prepare the relative concentrations of 0.2-0.005mg/ml by dilution method. The test concentrations of the extracts were prepared by dissolving 1mg of each in 1ml methanol. The relative concentrations of garlic acid prepared were placed into a 96- well- plate. 25µL of sample extract, 125µL of Lowry C were added, this was followed by 75µL of distilled water and 15µL of Folin ciocalteu reagents. The mixture was incubated for 40mins and the absorbance was taken at 750nm.

Preparation of Lowry Solution
Lowry A Solution: Lowry A solution is prepared by weighing 0.4g sodium hydroxide, and dissolving in 100ml volumetric flask with distilled water up to the mark of the flash. 2g of Na_2CO_3 was weighed in a separate 100ml volumetric flask and filled up to the mark with 0.1M NaOH solution.

Lowry B Solution: 1g of $NaKC_4H_4O_6$ (sodium potassium tartrate) was weighed and dissolved in 100ml Volumetric flask making 1% $NaKC_4H_4O_6$. 0.5g $CuSO_4$ was weighed in another 100ml volumetric flask and then filled up to the mark with 1% $NaKC_4H_4O_6$ solution.
Lowry C Solution: Lowry C solution was prepared from 50ml of Lowry A and 1ml of Lowry B.

HPLC Analysis
0.01g of the sample was weighed and dissolved in 1ml of methanol which was then subjected to HPLC measurement.

Results of Extraction and Fractionation

The weight of ethanol extract was 6.469g, and the weight of chloroform extract was 3.921g.

Percentage scavenging activity of the extracts and the standards for DPPH assay

Table 1: Scavenging activity of various extracts and standards

Concentration (µg/ml)	Vit C %	BHT %	Crude ethanol Extract %	Methanol Extract %	Pet Ether Extract %	Black Tea %
1000	98.4169	99.1557	50.1279	45.2921	58.5225	89.1206
500	97.3028	96.8748	25.1724	36.6531	46.9459	78.1899
250	96.7986	96.37006	21.2481	24.1857	35.7897	73.7312
100	96.8221	89.6980	18.5138	19.9049	29.6724	63.3404
10	18.6632	46.0334	29.5330	16.8625	40.4541	39.5119

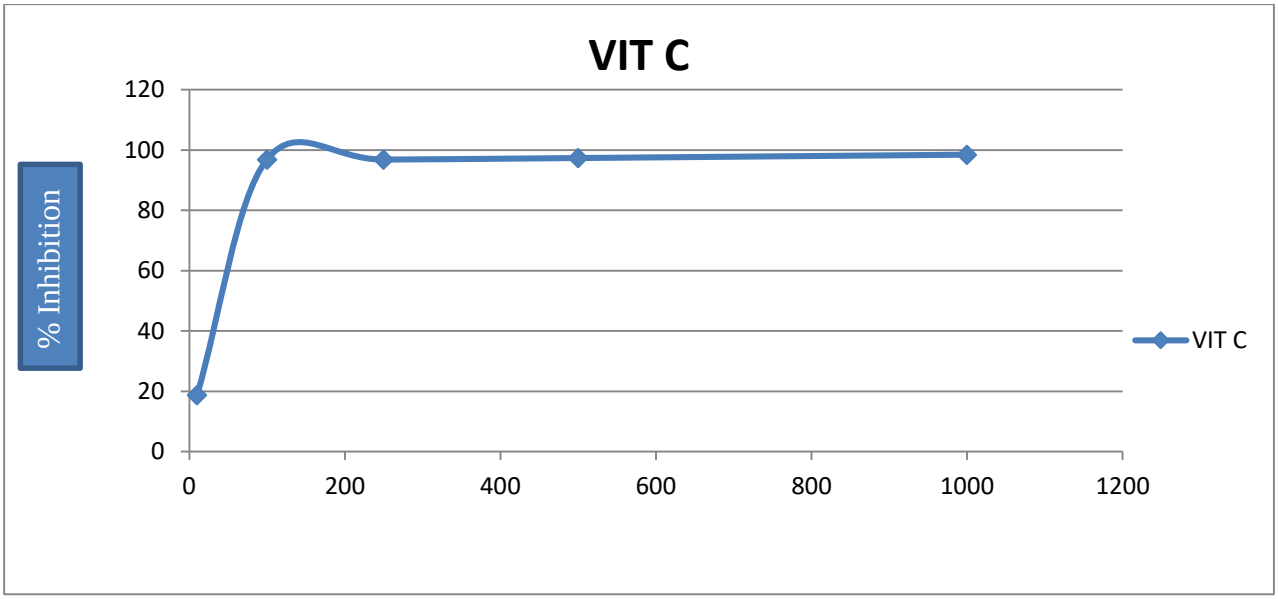


Figure 2: Scavenging activity at different concentrations of vitamin c

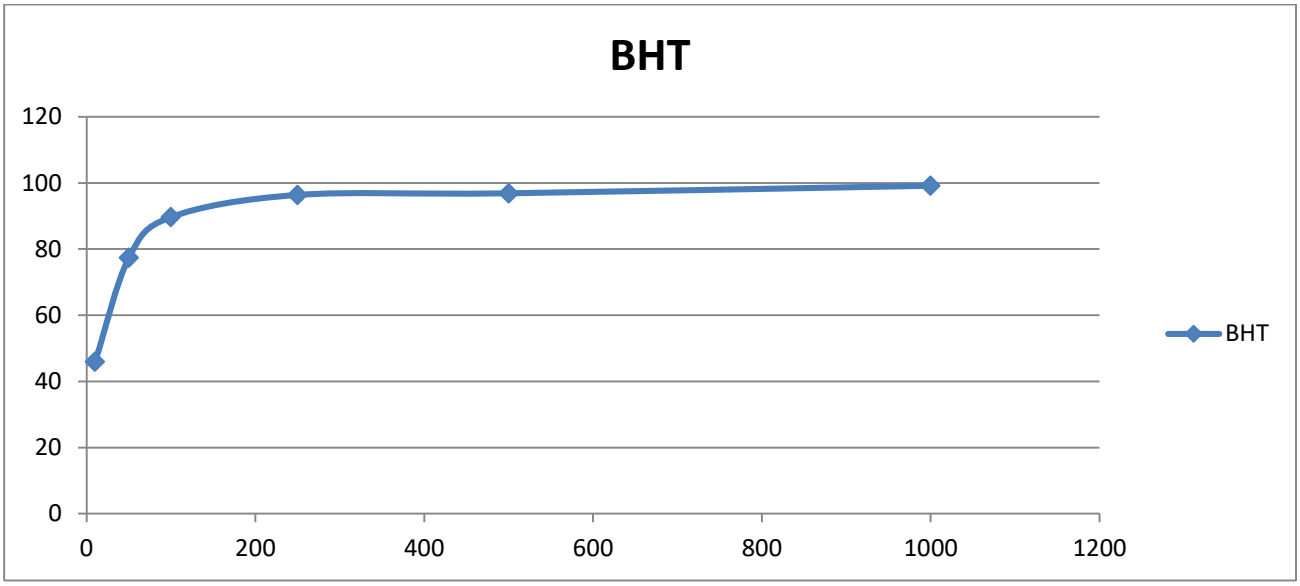


Figure 3: Scavenging activity at different concentrations of BHT

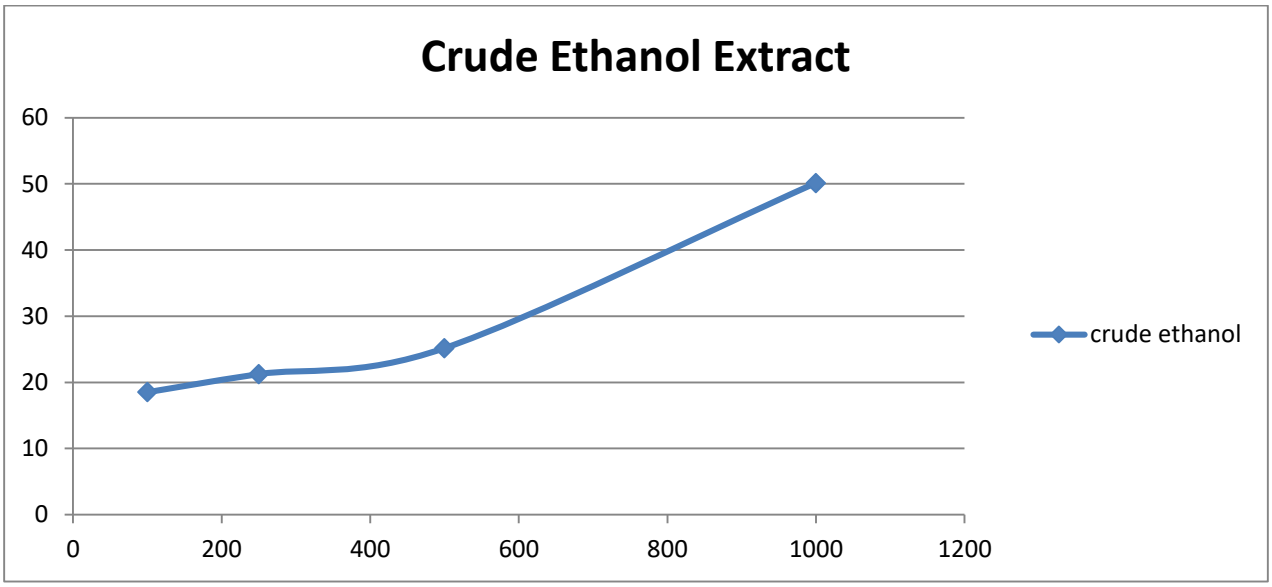


Figure 4: scavenging activity of different concentration of the crude ethanol extract.

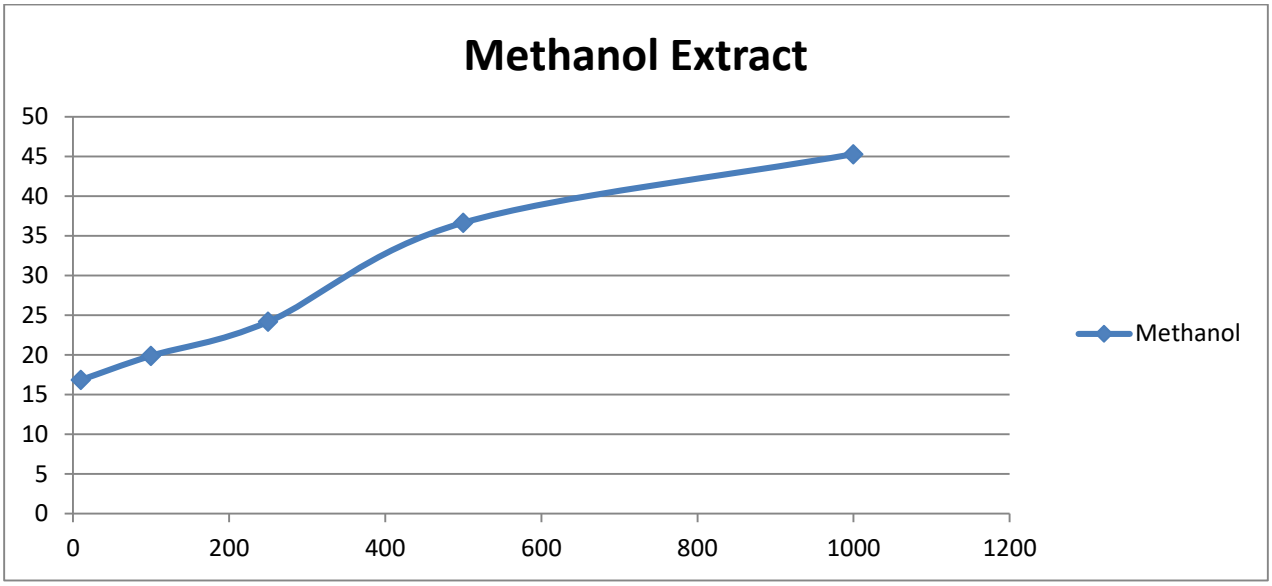


Figure 5: Scavenging activity at different concentrations of the Methanol extract

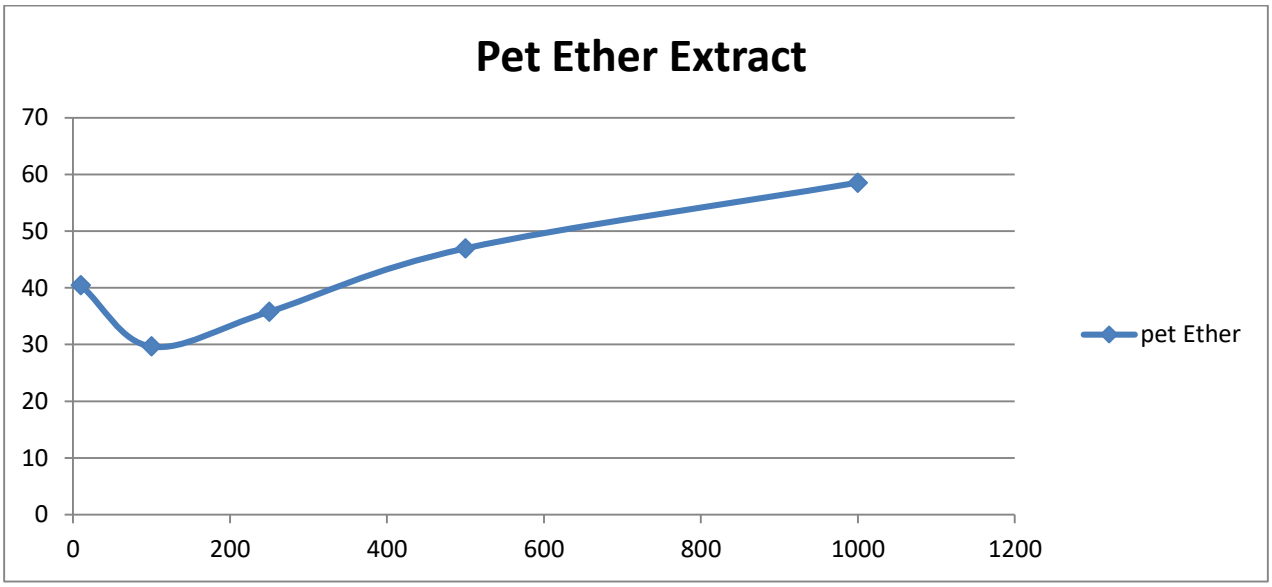


Figure 6: Scavenging at different concentration of the petroleum Ether extract

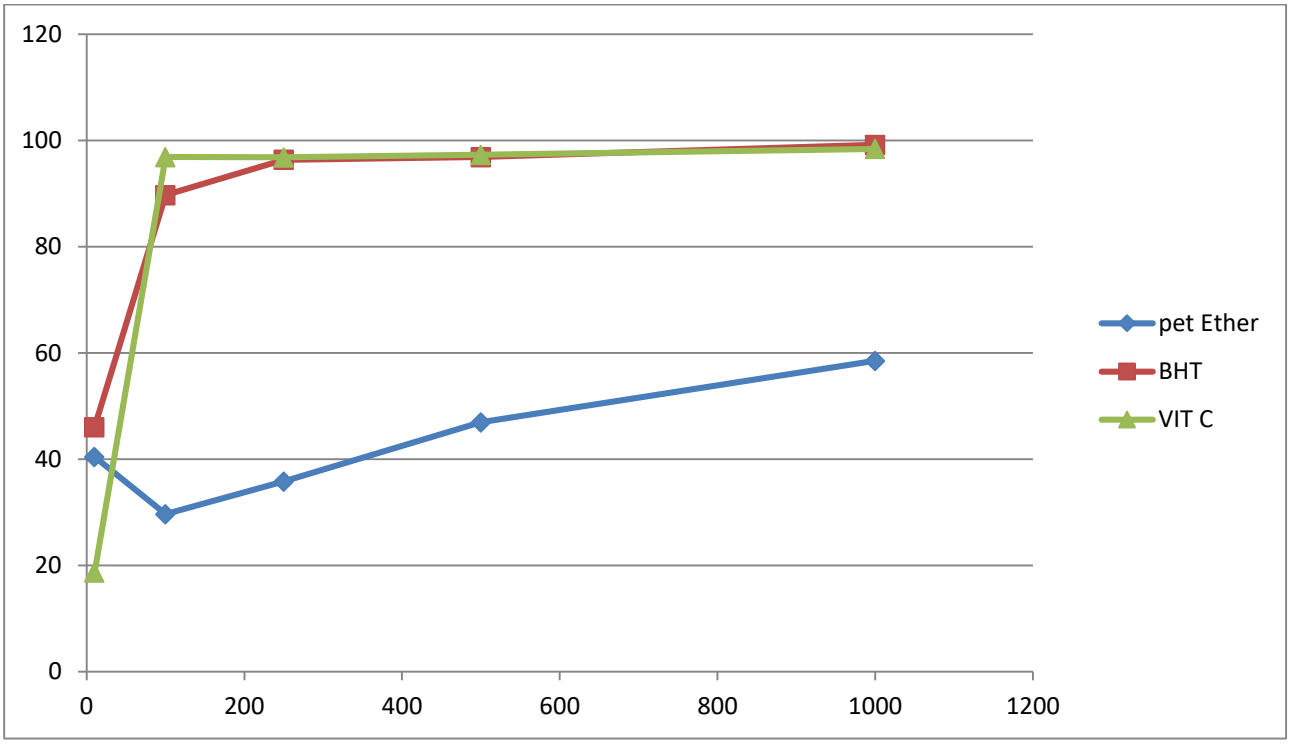


Figure 7: scavenging activity of pet Ether extract in comparison with vitamin C and BHT.

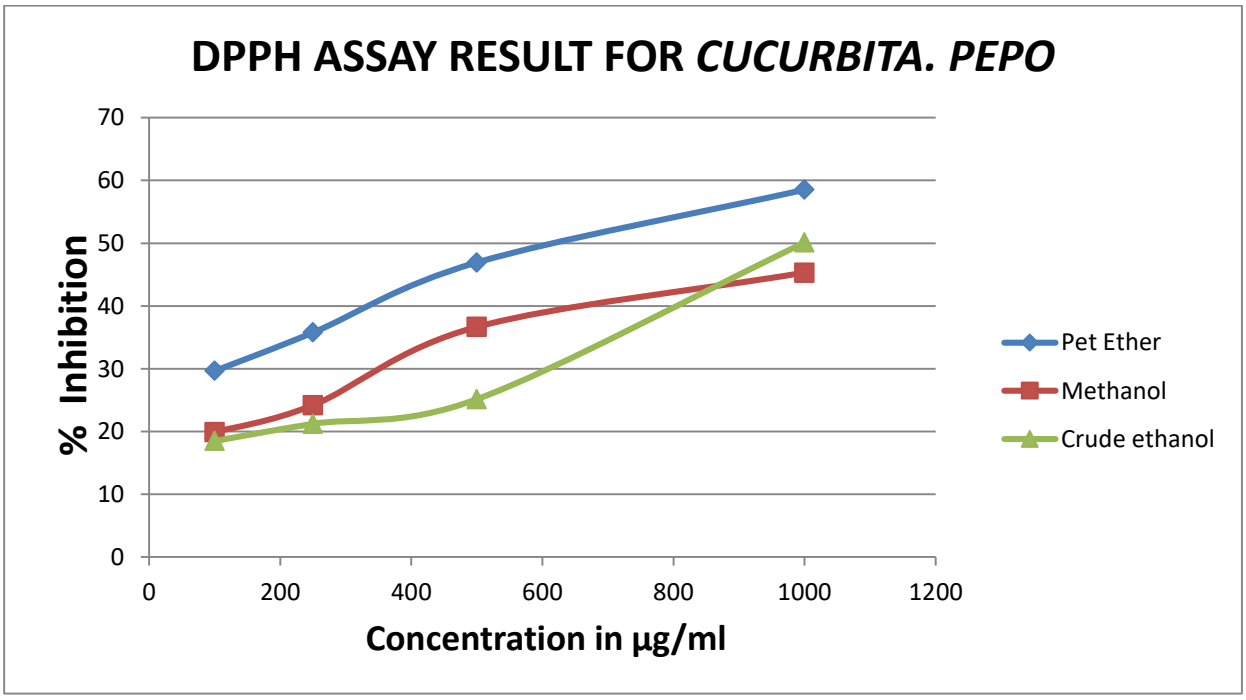


Figure 8: Comparison of scavenging activities of the three samples of *Cucurbita. pepo*

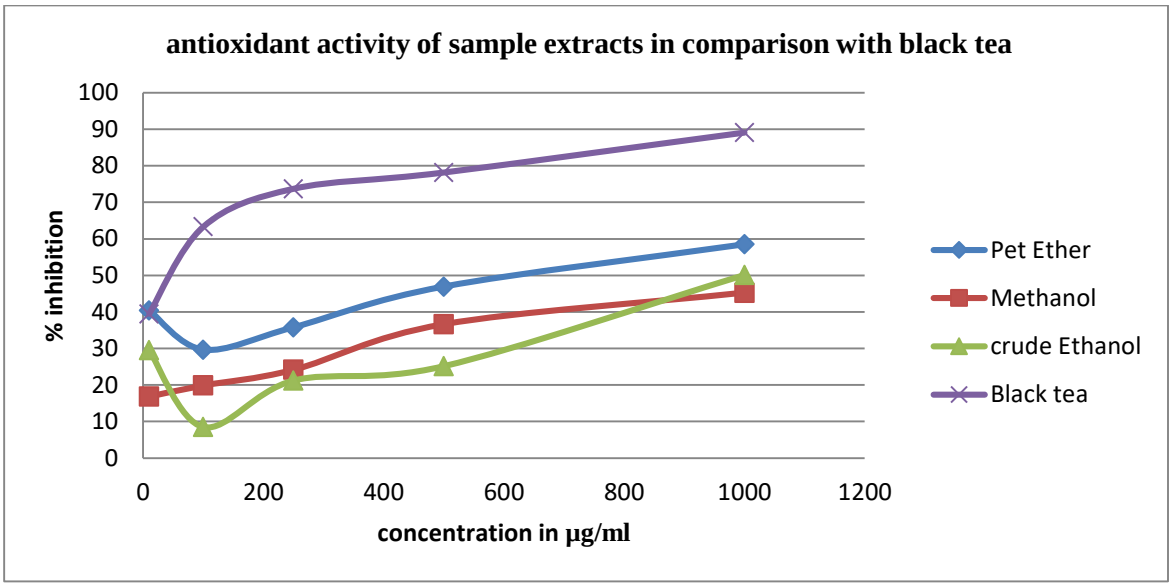
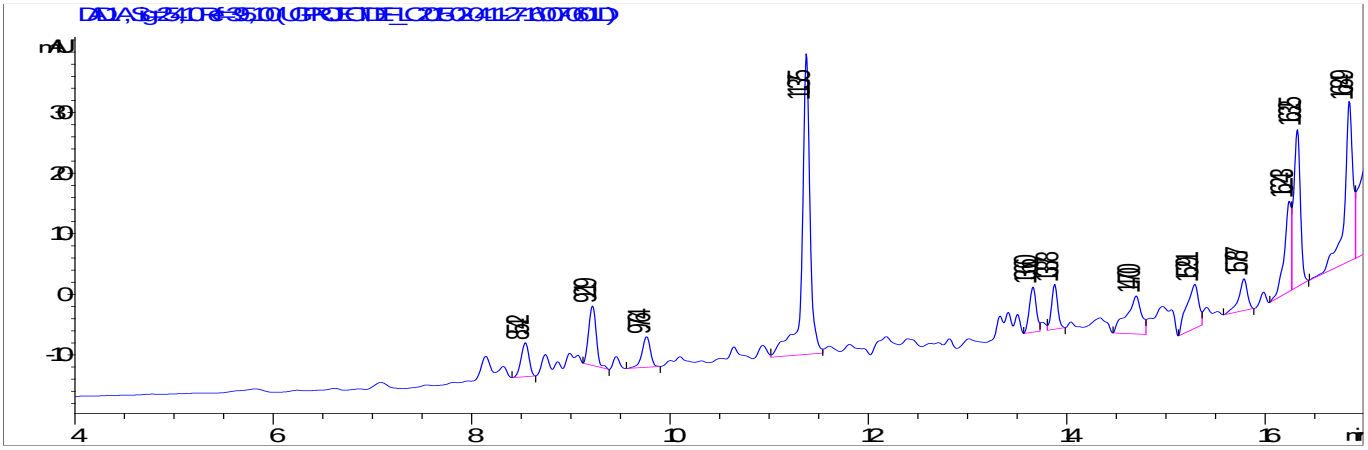


Figure 9: scavenging activity of various extracts compared with Black Tea

The antioxidant activity was observed after extraction, which was shown that vitamin c has higher antioxidant activity than that of the sample extract. Lower then pet ether extract.The result of DPPH assay is expected to give the colour change and after extraction colour change from purple to yellow. The comparison between *Cucurbita pepo* samples and black tea shows that Black tea has higher activity then *Cucurbita pepo.* Comparison between vitamin C and samples shows that vitamin c is more active than *Cucurbita pepo* samples.

HPLC Chromatogram Showing Peaks at Different Retention Times

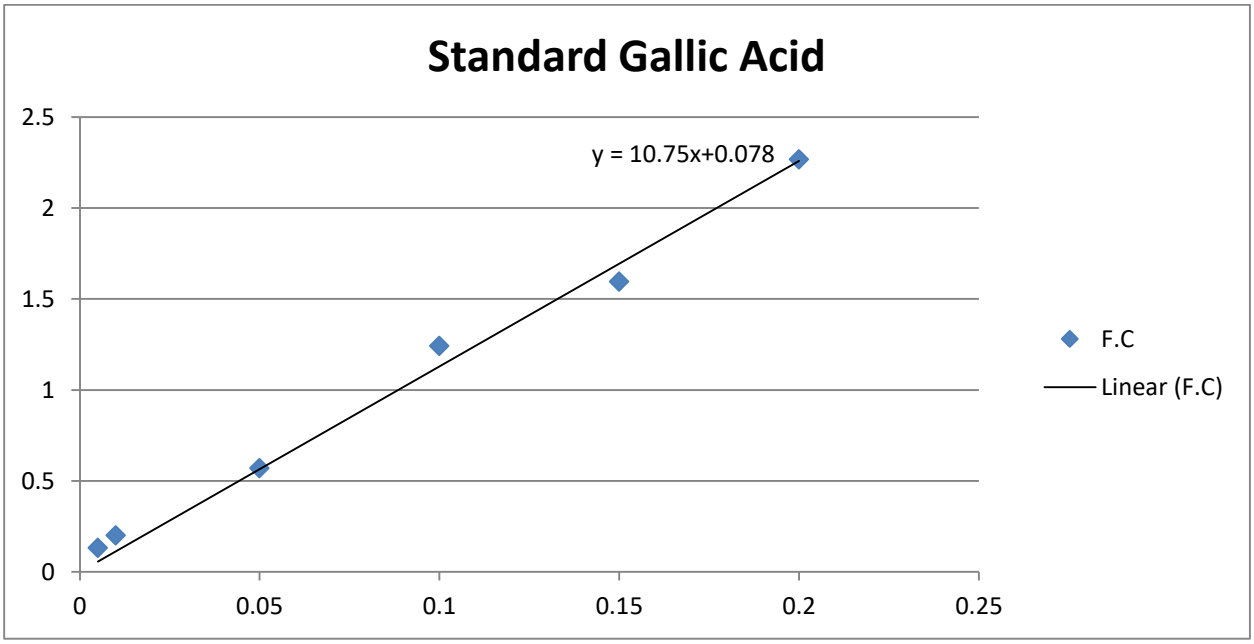


Pumpkin MeOH Extract HPLC Chromatogram

From the HPLC chromatogram, the result establishes the presence of compounds at 254nm and at different retention times which testify that the chromatogram closet to the origin was more polar than those far away.

Total Phenolic Content

Standard Calibration Curve of Gallic Acid.



Absorbance of Gallic Acid Equivalent to Extract.

CRUDE EXTRACT	Absorption(mg/g) of Gallic Acid Equivalent
Ethanol	8
Methanol	12.6
Pet Ether	17.1

Total Phenolic content indicated that the fraction of pet ether with 17.1mg/g of gallic acid equivalent has the highest number of phenols followed by methanol extract 12.6mg/g and ethanol fraction is the lowest with 8mg/g.

CONCLUSION

From the results obtained, *cucurbita pepo* leaves are found to be a source of natural antioxidant due to the antioxidant activity determined. The comparison between *Cucurbita. pepo* samples and vitamin C shows that they all have antioxidant activity but vitamin c is more active than the cucurbita *pepo* samples. HPLC Analysis shows the presence of different compounds occurring at different retention times. Folin ciocalteu reagent was used to determine the total phenolic content present in the *cucurbita pepo* samples.

References

Aiyegoro OA, Okoli AI. (2010). Preliminary Phytochemical Screening and in vitro antioxidant activities of the aqeous extract of *Helichrysum longifolium* DC. *BMC complementary and Alternative Medicine*;10**(12):** Pp1-8.

Ames, B.N, shigenaqa, M.K, Hagen, T.M. (1993). Oxidants, antioxidants and the degenerative diseases of Aging Proceeding of the National Academy of Sciences of the United State of America. 90, Pp 7915-7922.

AVRDC. (1990). Asian Vegetable Research and Development Center. Vegetable Production Training manual. Shanhua, Tainan. P447. Reprinted 1992.

Cadenzas, E and Packer, L.(eds). (1996). Hand Book of Antioxidants, plenum, New York.

Caili, F, Huan, S., and Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin plant. *Foods of Human nutrition.* 61, Pp73-80.

Devasagayam, T.P..A., Tilak, J.C, Boloor, K.K., Sane, K.S., Ghaskadbi, S., Lele , R.D. (2004). Free radicals and antioxidants in Human health. current status and future prospects. *J. Assoc. Physicians India.* Pp 794-804.

Lutskii V.I, Gromova A.S and N.A. Tyukavkina N.A. (1971). Aromadendrin, apigenin, and keampferol". *Chemistry of Natural Compounds.* 7**(2):** Pp197

Moon, J. K., and Shibamoto, T. (2009). Antioxidant Assays for Plant and Food Components. *J. Agric. Food Chem* ;57**(5)**: Pp1655-1666.

Nicoli, M.C., Anese, M., and Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in food and technology, Elservier Science Ltd* 10, Pp 94-100.

Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A. *et al.,* (1996). Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular diseases. *New-England journal of Medicine.* 334**(18):** Pp1150- 5.

Rice-Evans, C., and Miller, N.J. (1995). Antioxidants. the case of fruit and vegetables in the diet. *British food journal.* 97: Pp 35-40.

Shi *et al*., X. (2013). *LWT- Food science and Technology* 51: Pp433-440.

Sies, H. (ed). (1996). *Antioxidants in Diseases, Mechanisms and Therapy*. Academics Press, New York.

Smith, B.D. (1997). The initial domestication of *Cucurbita. pepo* in the Americas 10,000 years ago. *Science.* 276: Pp932-934.

Trachootcham, D., Alexandre, J., Huang, P. (2009). Targeting cancer cell by ROS (Reactive Oxygen Species) mediated mechanisms: A radical therapeutic approach, *Nat Rev. Drug, Discov*. 8579591.

Vander M. S., Chihande D., Zinangaf. (1997). The Role of Traditional Vegetables in Zinbabwe. Report for Community Technology Development Trust Supported by IDRC.

Yuan, B., Yoshino, Y., Kaise, T., Toyoda, H. (2011). Application of Arsenic Trioxide Therapy for Patient with Leukaemia In: sun HZ. editor. Biological Chemistry of AS, Sb and Bi. *John Wiley and Sons,* New York, Pp 263-292.