Estimation of total carotenoids and free radical scavenging activity of selected vegetables

Joseph O. Oiseoghaede[1] and Olukemi A. Odukoya[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos.
College of Medicine Campus, Idi-Araba, Lagos, Nigeria.

Corresponding author: Joseph O. Oiseoghaede
E-mail: joseph.oise14@gmail.com Phone: +234 8098253426

ABSTRACT

Background: Vegetables are known for their rich carotenoid content responsible for their antioxidant properties.

Objectives: This study sought to estimate the total carotenoid content (TCC) in four edible Nigerian vegetables, compare their free radical scavenging activity (FRSA) and relate their TCC to their respective FRSA.

Methods: The method employed for extraction involved homogenization with ethanol using a blender. Estimation of the TCC was obtained by an equation derived from their Chlorophyll A and B contents from reading absorbances of homogenates at 470nm, 649nm and 665nm. FRSA of ethanolic homogenates on 2,2-diphenyl-1-picrylhydrazyl (DPPH) by decreased absorbance of 1mM DPPH on adding extracts was read at 517nm. The FRSA obtained by 0.2-1% concentrations done in triplicates was compared statistically (ANOVA Test, $p<0.01$). Mean Inhibitory concentration (IC_{50}) value was obtained graphically via percentage inhibition/concentration curve.

Results: *Capsicum* annum had the highest TCC compared to the other vegetables. *Carica papaya* leaves exhibited a superior FRSA compared to other plants ($p<0.0001$). Results showed that there may be a direct relationship between TCC and FRSA. However, *Carica papaya* which had the highest FRSA did not show a high TCC. Hence, *Carica papaya* may possess other phytochemicals other than carotenoids responsible for its high FRSA.

Conclusion: This study shows that *Carica papaya* leaves had a high FRSA and antioxidant capacity due to the presence of carotenoids and other beneficial phytochemicals. This evidence supports the fact that it can be included into our diet as Africans.

Keywords: Antioxidant, total carotenoids, free radical scavenging activity, pawpaw.

Evaluation des caroténoïdes totaux et activité de libre balayage de végétaux sélectionnés

Auteur correspondant: Joseph O. Oiseoghaede
E-mail: joseph.oise14@gmail.com Phone: +234 8098253426

RESUME

Contexte: On reconnait chez les végétaux leur riche teneur caroténoïde responsable de leurs propriétés antioxydantes.

Objectifs: Cette étude a cherché à évaluer la teneur caroténoïde totale (TCT) dans quatre végétaux comestibles du Nigéria, comparer leur activité de libre balayage radical (FRSA) et établir un lien entre le TCC et leur FRSA respectifs.

Méthodes: La méthode utilisée pour l'extraction a impliqué l'homogénisation à l'éthanol à l'aide d'un mixer. L'évaluation de la TCT fut obtenue par une équation dérivée de leurs teneurs en chlorophylle A et B à partir des absorbances de lecture des résultats de l'homogénéisation à 470nm, 649nm et 665nm. La FRSA des résultats de l'homogénéisation éthanolique sur 2,2-diphenyl-1-picrylhydrazyl (DPPH) par l'absorbance réduite de 1mM DPPH sur les extraits ajoutants fut lue à 517nm. La FRSA obtenue par concentrations de 0.2-1% faites en triple exemplaires fut comparée statistiquement (Test ANOVA, $p<0,01$). La valeur de la concentration Inhibitrice Moyenne (IC_{50}) fut obtenue graphiquement par le pourcentage d'inhibition/courbe de concentration.

Résultats: Capsicum annum avait la plus forte TCT par rapport aux autres végétaux. Les feuilles de Carica papaya ont indiqué une FRSA supérieure par rapport aux autres plantes ($p<0,0001$). Les résultats ont indiqué qu'il peut y avoir une relation directe entre la TCT et la FRSA. Cependant, Carica papaya qui avait la plus forte FRSA n'a pas indiqué une forte TCT. D'où, Carica papaya pourrait posséder d'autres produits phytochimiques en dehors des caroténoïdes responsables de sa forte FRSA.

Conclusion: Cette étude montre que les feuilles de Carica papaya avaient un
e forte FRSA et une capacité antioxydante grâce à la présence des caroténoïdes et autres avantages phytochimiques. Cette évidence soutient le fait qu'elle peut être inclue dans notre régime alimentaire en tant qu'Africains.

Mots-clés: Antioxydant, caroténoïdes totale, libre activité de balayage radical, papaye.

INTRODUCTION

The art of using our food as cure for ailments is as old as mankind itself. Plant materials were among the treatments for diseases in the early days of man. The effects of plants materials could have been found through trial and error and may have led to wellness and if such plant is poisonous, death. Therefore, with the experience garnered by our early ancestors, edible plants were designated as food and medicine and poisonous plants were avoided.[1]

Scientists of 2nd century BC in a response to an increase in illnesses came to a conclusion that "it is the wholesome use of food that promotes the health of a person and that which is unwholesome is the cause of disease". The implication of this statement boils down to the fact that the origin of the vast majority of our health problems is what we put in our bodies every day. Therefore, if we eat healthy, we would be healthy and healthy food intake can help fight diseases.[2] Thus, over a long period in human history, use of food has been advocated to fight diseases.

Free radicals have been implicated as agents that contribute to progression of chronic diseases. Free radicals can be defined as atoms or molecules containing one or more unpaired electrons in their orbitals. They are formed continuously in body cells as a consequence of both enzymatic and non-enzymatic reactions. It has been estimated that the average person has around 10000–20000 free radicals attacking each body cell each day.[3] Some free radicals are good in that they enable the human body to fight inflammation, kill bacteria, and control the tone of smooth muscles, which regulate the working of internal organs and blood vessels. Conversely, increased or uncontrolled free radical activity might combine with other factors to cause some diseases such as neurodegenerative diseases, heart disease, cancers and so on. The balance between the production of free radicals and the antioxidant defences in the body has important health implications. Under the normal conditions the antioxidant defense system within the body can easily handle free radicals that are produced. If there are too many free radicals produced and too few antioxidants, this may cause chronic damage.[3]

Therefore, to maintain the balance of free radicals and antioxidants, we must ensure that we take in enough food rich in antioxidants to make sure that this balance remains in our favour. Foods from plants and animals are very rich sources of these natural antioxidants.[4]

Carotenoids are natural colourful plant pigments found in fruits and vegetables. Between 500 and 600 specific carotenoids have been identified of which only about 24 commonly occur in human foodstuff. The principal carotenoids of food are β-carotene, β-cryptoxanthin, Lycopene, Lutein and Violaxanthin. In local Nigerian diet, sources of carotenoids include Fluted gourd, Telfairia occidentals Hook leaves which has high content of β-carotene [5], Pawpaw or *Papaya Carica papaya* Linn. leaves [6], Red pepper *Capsicum annum* Linn. fruits [7] and Carrot *Daucus carota* Linn. roots. [8]

Carotenoids carry out their antioxidant activity by free radical scavenging. Free radical scavenging is achieved by different mechanisms such as quenching of singlet oxygen, addition, electron transfer and hydrogen atom transfer. [9] Thus, they reduce the amount of free radicals in the cells and hence prevent instigation of illnesses. Knowledge exists that show that carotenoid-rich vegetables are invaluable in prevention of chronic illnesses. This study was to estimate the total carotenoid content (TCC) in four edible Nigerian vegetables, compare their free radical scavenging activity (FRSA) and relate their TCC to their respective FRSA.

METHOD

Collection and identification of plant materials used: The plant materials were sourced from the market and their natural habitats in May 2013. The plants were identified and authenticated at the Department of Botany, University of Lagos. Museum specimens were kept at the herbarium. The voucher numbers were designated as:

Carica papaya Linn. (Caricaceae), LUH 5716
Daucus carota Linn. (Apiaceae), LUH 5717
Capsicum annum Linn. (Solanaceae) LUH 5919
Telfairea occidentalis Hook. (Cucurbitaceae) LUH 5720

Extraction procedure: The plants were extracted by homogenization. [10] The carotenoids were obtained from the plant material in the fresh form for the leaves of the fluted gourd and pawpaw and root of carrots. Fresh leaves of fluted gourd and pawpaw and roots of carrots were washed and rinsed with water. The plant material was cut using a table knife into smaller sizes. Thereafter, 500 g of the plant were put into a Moulinex blender and homogenized with 500 mLs of Absolute Ethanol as the solvent. The blended plant material was then filtered using a glass funnel with muslin cloth then cotton wool to obtain the filtrate and stored in amber bottles in the refrigerator until used.

Estimation of chlorophyll a, chlorophyll b and total carotenoids: The extracts stored in the amber bottles were brought out from the refrigerator. Due to the sensitivity of the UV spectrophotometer, the extracts

have to be well diluted until a clear solution was obtained. Finally, a 1 in 25 dilution was done for the stock solution to obtain a clear solution. This procedure was done in triplicates for the different plant extracts. An equation for the determination of total carotenoids and chlorophylls a and b in extracts in different solvents including Absolute ethanol was employed. [11] Absorbances of the diluted extracts already in triplicates were taken at 470 nm, 649 nm and 665 nm and values recorded. The concentrations (µg/ml) of the Chlorophyll a (C_a), Chlorophyll b (C_b) and Total carotenoids (C_{x+c}) were determined using the formulae stated below:

$C_a = 13.95\,A_{665} - 6.88A_{649}$

$C_b = 24.96A_{649} - 7.32A_{665}$

$$C_{x+c} = \frac{1000A_{470} - 2.05\,C_a - 114.8\,C_b}{229}$$

$A_{470,}$ $A_{649}$and A_{665} represent the absorbance readings that were taken at 470 nm, 649 nm and 665 nm respectively. The result obtained was given as µg/mL. To calculate the amounts of pigments present per gram (µg/g), the result obtained was multiplied by 25 which is the dilution factor. This gives the amount of pigment per 1 g of plant material.

Diphenylpicrylhydrazyl (dpph) free radical scavenging activity: The method employed was used in a similar study. [12]

0.0079 g of DPPH was weighed on a chemical balance and transferred into a 200 mL volumetric flask. Sufficient amount of Absolute ethanol was then added and crystals dissolved in it to obtain the 1mM DPPH.

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used for a rapid determination of qualitative antioxidant activity by its free radical-scavenging activity of the extracts. This is evident by the disappearance of the purple colour of DPPH. Briefly, to a 2 mL ethanolic solution of extract of various concentrations at 0.2%, 0.4%, 0.6%, 0.8% and 1% of the stock of the raw homogenates (1 g/mL) of the fresh plant materials was added 1ml of 1mM DPPH. A blank solution was prepared containing 2 mL of ethanol and 1 mL of 1mM DPPH. The experiments were carried out in triplicates. The test tubes were incubated for 15 minutes. The absorbance was read at 517nm. The free radical scavenging activity was calculated using the following formula:

% Inhibition of DPPH = $\{(A_B - A_A)/A_B\}$x100 where A_B is the absorption of blank sample and A_A is the absorption of tested homogenate solution. The results were expressed as percentage inhibition of DPPH.

Determination of IC_{50} values: Mean Inhibitory concentration (IC_{50}) value is the concentration that causes 50% inhibition of DPPH free radicals and is a parameter widely used to measure antioxidant/free radical scavenging power. [9] It was obtained by using Graph Pad Prism Software (Prism 6) using linear equation of the line. On a plot of the percentage inhibition against the concentration of the extract, a trend line equation: y = ax + b were determined. The values were calculated by transforming the equation above and the expression x (concentration) at which y-value (percentage DPPH inhibition) is 50% was accepted as unknown. [13] The values were computed and meaningful inferences made.

Statistical analysis/evaluation: The results of the concentration of the chlorophyll A, chlorophyll B and total carotenoids in the plant samples were subjected to statistical evaluation by obtaining the mean values, standard deviation and standard error of the means of each sample used.

The observations of chlorophyll A, chlorophyll B and total carotenoids were expressed as Mean ± Standard error of mean(SEM), n=3.

The observations of the percentage of inhibition of DPPH were also expressed as Mean ± Standard error of mean(SEM), n=3.

Graph Pad Prism software (Prism 6) and Microsoft Excel were used to carry out the statistical analysis.

Two-way ANOVA Test was also used to compare the differences in percentage of DPPH inhibition at different concentrations among the different plant extracts. The tests were done at 99% (Alpha=0.01) confidence intervals and meaningful inferences drawn from them.

RESULTS

Chlorophylls A & B content and total carotenoid content: Results of the Chlorophylls A & B and TCC are represented in Tables 1 and 2.

DPPH free radical scavenging activity and IC_{50} determination

The results of the DPPH scavenging activity are expressed in Table 3.

Statistical comparison of DPPH inhibition of extracts: Statistical evaluation/ analysis of the different concentration of homogenates are expressed in Table 4.

IC_{50}values of plant extracts: IC_{50} value of each homogenate was also determined from linear plots as represented in Figure A. The IC_{50} values of each

TABLE 1: Mean chlorophyll A & B content

Plant	Chlorophyll A content (µg/mL) of extract	Chlorophyll A present µg /g of plant material	Chlorophyll B content (µg/mL) of extract	Chlorophyll B present µg /g of plant material
Daucus carota Linn. (Apiaceae)	0.4955±0.0127	12.3875	1.8821±0.0868	47.0525
Capsicum annum Linn. (Solanaceae)	0.3598±0.0421	11.9933	0.7292±0.0759	24.3067
Carica papaya Linn. (Caricaceae)	5.3570±1.0364	133.9250	5.5866±2.5383	139.6650
Telfairia occidentalis Hook (Cucurbitaceae)	3.5431±0.2483	88.5775	4.3608±0.3084	109.0200

Data expressed as Mean±SEM, n=3

Table 2: Estimation of total carotenoids

Plant	Total carotenoids (µg/mL) of extract	Carotenoids present µg /g of plant material
Daucus carota Linn. (Apiaceae)	0.0337	0.8425
Capsicum annum Linn. (Solanaceae)	3.0485	101.6167
Carica papaya Linn. (Caricaceae)	0.3497	8.7425
Telfairia occidentalis Hook (Cucurbitaceae)	0.2182	5.4550

Table 3: Mean DPPH free radical scavenging activity (% inhibition)

% Inhibition	0.2	0.4	0.6	0.8	1.0
Daucus carota Linn. (Apiaceae)	1.65±0.41	2.18±0.99	4.24±0.93	4.49±0.90	4.50±1.98
Capsicum annum Linn. (Solanaceae)	3.67±3.50	15.57±2.39	16.97±0.50	22.88±4.48	24.28±0.56
Carica papaya Linn. (Caricaceae)	15.27±6.18	45.30±3.43	52.22±2.09	64.22±1.34	71.12±2.53
Telfairia occidentalis Hook (Cucurbitaceae)	8.49±0.42	10.62±2.76	10.75±3.74	12.95±1.06	23.44±1.47

Data expressed as Mean±SEM, n=3

Table 4: Statistical comparison of DPPH inhibition of extracts

Concentration(%)	0.2	0.4	0.6	0.8	1.0
Carrot vs. pepper	3.67±3.50	15.57±2.39**	16.97±0.50**	22.88±4.48****	24.28±0.56****
Carrot vs. pawpaw	15.27±6.18**	45.30±3.43****	52.22±2.09****	64.22±1.34****	71.12±2.53****
Carrot vs. fluted gourd	8.49±0.42	10.62±2.76	10.75±3.74	12.95±1.06	23.44±1.47****
Pepper vs. pawpaw	15.27±6.18*(No)	45.30±3.43****	52.22±2.09****	64.22±1.34****	71.12±2.53****
Pepper vs. fluted gourd	8.49±0.42	10.62±2.76	10.75±3.74	12.95±1.06*(No)	23.44±1.47
Pawpaw vs. fluted gourd	8.49±0.42	10.62±2.76****	10.75±3.74****	12.95±1.06****	23.44±1.47****

(ANOVA analysis, 99% Confidence Interval, p<0.01)
Data expressed as Mean±SEM at 5 concentrations.
*(No)= Not significantly different from first extract at p<0.01 (p <0.05)
**= Significantly different from first extract at p<0.01(p<0.01)
**** = Significantly different from first extract at p<0.01(p<0.0001)

Table 5: IC_{50} values of plant extracts

Plant extract	IC_{50} values
Daucus carota Linn. (Apiaceae)	12.2325
Capsicum annum Linn. (Solanaceae)	1.9734
Carica papaya Linn. (Caricaceae)	0.6057
Telfairia occidentalis Hook (Cucurbitaceae)	2.8805

Table 6: Carotenoid content of plants in this study compared with literature

Plants	TCC in this study (µg/g)	TCC in recent works (µg/g)	References
DC	0.8425	295	8
CA	101.6167	187-10,121	14
CP	8.7425	38.6(β-carotene)	6
TO	5.4550	989(β-carotene)	5

DC= Daucus carota, CA= Capsicum annum, CP= Carica papaya, TO= Telfairia occidentalis

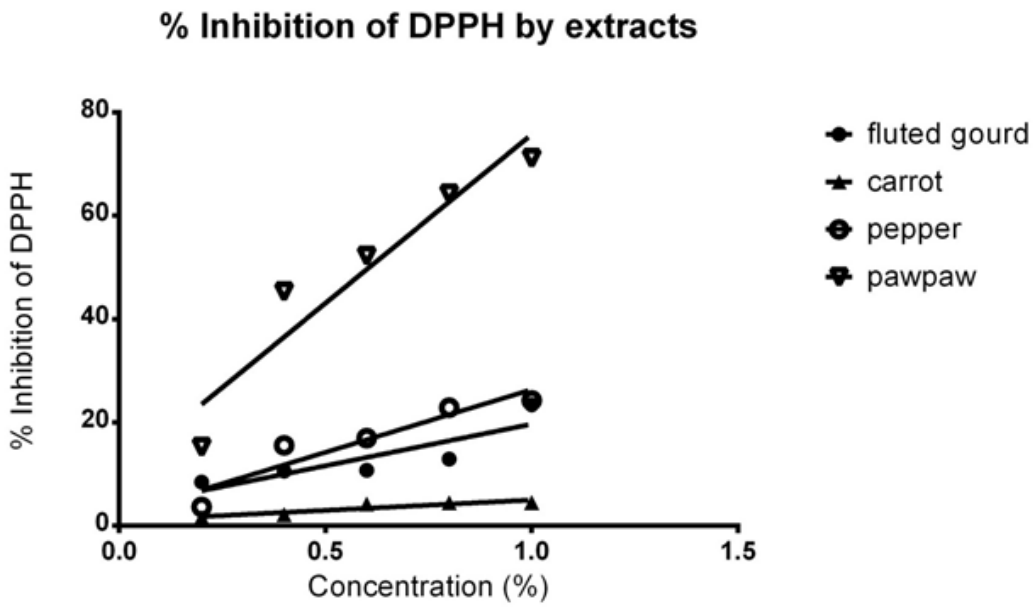

Figure 1: Determination of IC_{50} using linear plots

Equations of the linear plots

y = 16.12x + 3.581, R^2= 0.7433, Fluted gourd
y = 4.005x + 1.009, R^2= 0.8383, Carrot
y = 24.27x + 2.115, R^2= 0.8828, Pepper
y= 65.31x + 10.44, R^2= 0.9072, Pawpaw

DISCUSSION

Chlorophyll A & B content

From the results, *Telfairia occidentalis leaves* and *Carica papaya* leaves had the highest contents of chlorophyll. This was expected as they were green and fresh and still possessed chlorophyll. *Daucus carota* and *Capsicum* annum also had appreciable amounts of chlorophyll. This was because they contained the green pigments as some of their parts were unripe.

Estimation of total carotenoids

The results show that all the samples have appreciable amounts of total carotenoids. *Capsicum annum* had the highest TCC of 101.6167 µg per gram of plant material. *Daucus carota* had the lowest TCC of 0.8425 µg per gram of plant material. *Capsicum annum* had about 120 times more carotenoids than *Daucus carota*, 19 times more carotenoids than *Telfairia occidentalis* and about 12 times more carotenoids than *Carica papaya*.

This is consistent with work that was done by other workers as seen in the table 6. The TCC of the plants studied follow the order as stated below:

Capsicum annum > Carica papaya > Telfairia occidentalis > Daucus carota

The fact that this study also shows that *Capsicum annum* had the highest total carotenoid contents lends credence to the study. The reason for the higher carotenoid contents in *Capsicum annum* may be due to the presence of higher red pigmentation which indicates a higher amount of carotenoids notably Xanthophylls especially the red carotenoids, capsanthin and capsorubin (oxygenated carotenoids). [15]

It was generally observed that compared to previous studies, there was wide variation in total carotenoid contents of the plants. This could be due to the difference in content of secondary metabolites based on the fact that previous studies were done in different geographical regions in the world. TCC of these plants studied showed that they are rich sources of these beneficial phytochemicals and can serve as safe substitutes for synthetic antioxidants. [16]

Free radical scavenging activity

Scavenging activity was seen as the extracts were added to the DPPH by a visible disappearance of the purple colour to a brown or yellow colouration. At 1%, *Carica papaya* leaves scavenged DPPH more effectively than the other plants ($p < 0.01$). All the plants showed a dose dependent DPPH scavenging activity. That is, the higher the concentration of the extract, the more active the

extract became. *Carica papaya* leaf extracts was about three-fold more efficient than *Capsicum annum* fruit extract and *Telfairia occidentalis* leaf extract in scavenging DPPH free radicals. It was also about sixteen-fold more efficient than *Daucus carota* in scavenging DPPH free radicals. The FRSA of *Capsicum annum* and *Telfairia occidentalis* were statistically similar. *Carica papaya* leaf extracts had a statistically significant superior FRSA when compared to that of other plant extracts ($p \leqslant 0.01$). The FRSA of the plants studied follow the order as stated below:

Carica papaya > Capsicum annum = Telfairia occidentalis > Daucus carota.

Relationship between the TCC and FRSA

Carotenoids, as earlier discussed have free radical scavenging activity hence it can be assumed that the plant with the highest total carotenoid content should have the highest FRSA and the plant with the least total carotenoid content should have the least FRSA.

Daucus carota had the least total carotenoid content of 0.8425 µg /g of fresh plant material and are expected to have the least FRSA. This particular argument holds true for *Daucus carota* because it had the least FRSA of 4.5%. Hence, it could be assumed that the bulk of its FRSA is due to the carotenoids present in it.

Telfairia occidentalis leaves had the second lowest total carotenoid content of 5.4550 µg/g of fresh plant material and are expected to have the second lowest FRSA. The argument also holds true for *Telfairia occidentalis* leaves because it had the second lowest FRSA of 23.44%. It could also be assumed that the FRSA of the plant is related to its carotenoid contents.

However, *Carica papaya* leaves and dried *Capsicum annum* fruits seem to be the exception to this argument. Dried *Capsicum annum* fruits had the highest total carotenoid contents of 101.6167µg/g and *Carica papaya* leaves had the second highest total carotenoid contents of 8.7425µg/g but *Carica papaya* leaves had a higher FRSA of 71.12% compared to that of *Capsicum annum* which was 24.28%. Though the Total carotenoid contents and FRSA of *Capsicum annum* was higher than that of *Telfairia occidentalis* leaves and *Daucus carota*, it was not as high as that of *Carica papaya* leaves which had a lower carotenoid content. The reason for this observation is not farfetched. The DPPH FRSA test is not a specific test for the Carotenoid FRSA hence it is possible that the *Carica papaya* leaves extracts had other anti-oxidants more than the other plants hence its higher FRSA compared to the other plants. Therefore, the earlier stated argument of the higher the total carotenoid content, the higher the FRSA does not hold true for *Carica papaya* leaves used in this study and may never hold true as there are other anti-oxidant metabolites in these plants. However, the earlier stated argument can be tested to hold true if a specific test or assay for the anti-oxidant property of carotenoids in these plants was used.

Ic_{50} values of plant extracts

IC_{50} value is the concentration that causes 50% inhibition of DPPH free radicals. This value is indicative of the FRSA of the extracts being tested. The extract with the lowest IC_{50} is has the strongest antioxidant capacity because a lower concentration is required to reduce the concentration of DPPH free radicals by 50%. [9]

According to the results, *Carica papaya* leaves had the lowest value of 0.6057 making it the plant with the strongest anti-oxidant activity. This was followed by dried *Capsicum annum* fruits with value of 1.9734 and Fluted gourd leaves with a value of 2.8805. *Daucus carota* have the highest value of all the extracts with a value of 12.2325.

Since the lowest value indicate higher activity, the order based on activity, will therefore be:

Carica papaya > Capsicum annum > Telfairia occidentalis > Daucus carota.

This test showed that a smaller amount of *Carica papaya* leaves is required to exhibit antioxidant activity compared to the other plants. Hence, *Carica papaya* leaves are a good source of natural antioxidants. [17]

CONCLUSIONS

Total carotenoid content of the four plants in the study showed that the dried *Capsicum annum* fruits had the highest carotenoid content and *Daucus carota* had the lowest carotenoid content.

All four plants showed free radical scavenging property. *Carica papaya* leaves had the best FRSA and Daucus carota had the least FRSA. Hence, the good antioxidant activity of these vegetables can be harnessed as a suitable preventive against adverse effects of free radicals that are produced daily in our bodies if they are continuously included in our diet.

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REFERENCES

1. Sofowora A (1993). Medicinal plant and Traditional medicine in Africa. 3rd Edn. Spectrum books, Ibadan, p12.
2. Caldecott T (2011). Food as Medicine: The theory and Practice of Food. *PhytoAlchemy*, New York, pp 11-12.
3. Pala FS and Gurkan H (2008).The role of free radicals in ethiopathogenesis of diseases. *Advances in Molecular Biology*, 1: 1-9.
4. Rietjens IM (2002). The pro-oxidant chemistry of the natural antioxidants, Vitamin C, Vitamin E, carotenoids and flavonoids. *Environmental Toxicology and Pharmacology*, 11(3-4): 321-33.
5. Badifu GIO, Akpapunam MA and Mgbemere VM (1995). The fate of beta-carotene in processed leaves of fluted pumpkin (Telfairia occidentalis Hook.): A popular vegetable in Nigerian diet. *Plant Foods for Human Nutrition*, 48(2):141-147.
6. Maisarah AM, Amira BN, Asmah R and Fauziah O (2013). Antioxidant analysis of different parts of Carica papaya. *International Food Research Journal*, 20(3): 1043-1048.
7. Southon S and Faulks R (2003). Carotenoids in food: Bioavailability and functional benefits. In: Johnson I, Williamson G (Eds.), Chapter 7. Phytochemical functional foods. Woodhead Publishing Limited, CRC Press, Cambridge, UK, pp. 107-127.
8. Mech-Nowak A, Świderski A, Kruczek M, Łuczak I and Kostecka-Gugała A (2012). Content of carotenoids in roots of seventeen cultivars of Daucus carota L. *Acta Biochimica Polonica*, 59(1):139–141.
9. Qian H and Nihorimbere V (2004).Antioxidant power of phytochemicals from Psidium guajava leaf. J*ournal of Zhejiang University Science*, 5 (6): 676-683.
10. Dere S, Gunes T and Sivaci R (1998). Spectrophotometric Determination of Chlorophyll - A, B and Total Carotenoid Contents of Some Algae Species Using Different Solvents. *Turkish Journal of Botany* , 22: 13-17.
11. Lichtenthaler HK and Wellburn AR (1983). Determination of Total Carotenoids and Chlorophylls A and B of Leaf in Different Solvents. *Biochemical Society Transactions*, 11: 591-592.
12. Ayoola GA, Coker HAB, Adesegun SA, Adepoju Bello AA, Obaweya K, Ezennia EC and Atangbayila TO (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants used for Malaria. *Tropical Journal of Pharmaceutical Research*, 7 (3): 1019 - 1024
13. Sierzant K and Gabrielska J (2009). Estimation of antioxidative properties of the natural polyphenols in the oxidation process of model liposome membranes. Proceedings of International Ph.D. Students Conference Faculty of Agronomy, Mendel University of Agriculture and Forestry, Brno, Czech Republic. ISBN: 978-80-7375-352-8.
14. Wall MM, Waddell CA and Bosland PW (2001).Variation in β-Carotene and Total Carotenoid content in Fruits of Capsicum. *Horticultural Science* , 36(4): 746-749.
15. Jaswir I, Noviendri D, Hasrini RF and Octavianti F (2011). Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry. *Journal of Medicinal Plants Research*, 5(33): 7119 – 7131.
16. Kasim LS, Olaleye KO, Oladosu IA, Aiyelaagbe OO and Flamin G (2014). Antioxidant and Cytotoxicity of Essential oils from Struchium sparganophora (Linn) Ktze Asteraceae. *Journal of Pharmaceutical Sciences and Pharmacy Practice*, 10 (1): 12-15.
17. Aravind G, Bhowmik D, Duraivel S and Harish G (2013). Traditional and Medicinal Uses of Carica papaya. *Journal of Medicinal Plants Studies*, 1(1): 7-15.