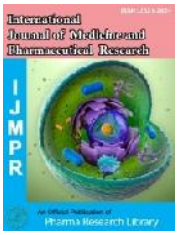
International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr



Research Article **Open Access**

Anticancer Activity of Crude Extract and Carotenoid Pigments from Vegetables

Ms. Ancilla Senoretta B. & Dr. V. Judia Harriet Sumathy*

Postgraduate & Research Department of Biotechnology, Women's Christian College, Chennai – 600 006.

A B S T R A C T

Vegetables can be eaten either raw or cooked and play an important role in human nutrition. Being mostly low in fat and carbohydrates, but high in vitamins, minerals and fiber they contain important antioxidant such as vitamins A, C and E. When vegetables are included in the diet, there is found to be a reduction in the incidence of cancer, stroke, cardiovascular disease and other chronic ailments. Research has shown that compared with individuals who eat less than three servings of fruits and vegetables each day, those that eat more than five servings have an approximately twenty percent lower risk of developing coronary heart disease or stroke. Vegetables contain a great variety of other phytochemicals (bioactive non-nutrient plant compounds), some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anti carcinogenic properties. Natural antioxidants such as vitamin C, tocopherols, flavonoids and other phenolic compounds are known to be present in certain plants**.** Recent studies have shown the importance of vegetables in a healthy diet in preventing degenerative diseases caused by oxidative stress. Vitamins and phytochemicals, such as ascorbic acid, carotenoids, polyphenols, and fiber have been regarded as the bioactive substances responsible for these effects and as spinach shows all these qualities it is highly recommended to add a daily intake of it. The present study is aimed at studying the Anticancer activity of Crude Extract and Carotenoid pigments of certain selected vegetables.

Keywords: Vegetables, Carotenoids, Phytochemicals, Spinach and Anticancer activity.

A R T I C L E I N F O

CONTENTS

Article History: Received 18 August 2016, **Accepted** 25 September 2016, **Available Online** 10 October 2016

***Corresponding Author**
Dr. V. Judia Harriet Sumathy
Postgraduate & Research Department of Biotechnology, Women's Christian College, Chennai–600 006.
Manuscript ID: IJMPR3167

PAPER-QR CODE

Citation: V. Judia Harriet Sumathy. Anticancer Activity of Crude Extract and Carotenoid Pigments from Vegetables. *Int. J. Med. Pharm. Res*., 2016, 4(5): 276-280.

1. Introduction

Carrots are one of the best sources of β-carotene. The carotene content of carrots ranges from 60–120 mg/100 g, but some varietes can contain up to 300 mg/100 g **(Velíšek, 1999)**. Carotens contain mainly β-carotene, i.e. about 80% **(Jeszka, 1997)**. The pigments are bound by proteins (**Seow-Mun Hue *et. al.*, 2011)**. Carrots can provide a significant amount of vitamin A **(Ana P. Tiveron *et. al.*, 2012).** Beet root (*Beta vulgaris* L.) ranks among the 10 most powerful vegetables with respect to its antioxidant capacity. Beet root is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which comprise two main groups, the red betacyanins and the yellow betaxanthins **(Joanna Fiedor and Kvetoslva Burda, 2014)**. Betalains have been extensively used in the modern food industry. Betalains, because of their relative scarceness in nature, have not been much explored as bioactive compounds, but some studies have indicated their potential as antioxidant pigments **(P. R. Onkar, 2013).** Dark leafy greens like spinach are important for skin and hair, bone health by providing protein, iron, vitamins and minerals. The possible health benefits of consuming spinach include improving blood glucose control in diabetics, lowering the risk of cancer, lowering blood pressure, improving bone health, lowering the risk of developing asthma and more **(P.M.Dey and J.B. Haarborne, 1997).** Spinach is also one of the best sources of dietary magnesium, which is necessary for energy metabolism, maintaining muscle and nerve function, heart rhythm, a healthy immune system and maintaining blood pressure **(Delia -Gabriela Dumbravă *et. al.*, 2010).**

Figure 1: Dried Vegetable samples

Magnesium also plays a major part in many biochemical reactions that occur in the body. Those with digestive disorders, alcoholic, older adults and individuals taking medications such as antibiotics and diuretics are more likely to have a magnesium deficiency and should consume more leafy greens (**Saiful Islam *et. al.*, 2011).** Spinach also contains vitamin K, fiber, phosphorus and thiamine (www.medicalnewstoday.com**).** Carotenoids are known to suppress the growths of tumors in in vitro (test tube) and in vivo (animal) studies **(S. Sonia, K, *et. al.*, 2007)**. The various carotenoids such as lycopene, β-carotene, α-carotene, lutein and canthaxanthin can decrease malignant transformation of cells (**Rajeswari Satapathy and Paramjyoti Swamy, 2012)**. There have been positive reports on dietary carotenoids improving fertility or reproduction capacity in a number of animals **(A. Bendich, 1989) (Figure 1).**

Carotenoids are also suggested to participate in: (i) the stimulation of the immune system; (ii) the modulation of intracellular signaling pathways (gap junction communication); (iii) the regulation of the cell cycle and apoptosis; (iv) the modulation of growth factors; (v) cell differentiation; and (vi) the modulation of various types of receptors or adhesion molecules and many other physiologically significant processes. Thus carotenoids are pigments that are found to have multimedicinal value. The present study is aimed at isolating carotenoid pigments from various **Vegetables** such as Carrot, Beet root, Red spinach and Green spinach which are rich in vitamin A, vitamin C and beta carotene and to evaluate and compare its Anticancer activity.

2. Materials and Methods

Samples used in the Present Study are as Follows

Green spinach (*Sauropus androgynus* (L.)Merr.)
Beetroot (*Beta vulgaris* L.)
Red spinach (*Amaranthus dubius* Mart.ex Thell.)
Carrot (*Daucus carota* L.)

Preparation of Extracts:

The Vegetables were collected and dried in shade for few weeks. The dried samples were ground into powder. 5gm of the dried sample powder was weighed and immersed in 50 ml of the solvents – Ethanol, Ethyl acetate and Chloroform for 48 hours. The carotenoid pigments were isolated using Column Chromatography and was quantified using the formula

Total carotenoid content (µg/g)= A xV(ml) x10^4 / $A^{1\%}$cm x W (g)

Where A is the absorbance of the carotenoid pigment at 450 nm, V is the total extract volume, $A^{1\%}$cm is the absorption coefficient of β carotene in hexane (2600), W is the sample weight. The samples were further subjected to Thin Layer Chromatography and FTIR studies. The Anticancer activity was carried out using MTT Assay Method

Anticancer Activity of the Extracts - MTT Assay

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an

organic solvent (eg. isopropanol) and the released, solubilized formazan reagent is measured spectrophoto metrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

Requirements

Cancer cell lines (MCF7)**,** 96 well plate**,** Dulbecco's Modified Eagle Medium, Foetal Bovine Serum, Antibiotics**,** MTT Reagent, Dimethyl sulphoxide. Cancer cell lines were purchased from Cancer Institute, Chennai. The cells were grown in a 96 well plate in Dulbecco's Modified Eagle Medium, supplemented with 10% Foetal Bovine Serum and antibiotics (Penicillin-G). About 200µl of the cell suspension was seeded in each well and incubated at 37^{o}C for 48 hours with 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various concentrations of the Vegetable extracts and their carotenoid extracts and incubated for 24hours. The cytotoxicity was measured using MTT (5mg/ml). After incubation at 37^{o}C in a CO_2 incubator for four hours, the medium was discarded and 200µl of DMSO was added to dissolve the formazon crystals. The absorbance was read in a micro plate reader at 570nm.

Cyto toxicity was calculated by the following formula:

Viability % = (Test OD/Control OD) x 100

Cell toxicity % = 100 – Viability %

3. Results and Discussions

Isolation of Carotenoid Pigments by Column Chromatography

Carotenoid pigments were effectively separated from the sample extracts separately in a silica gel column with 100% hexane. The yellow colour band which gets separated when eluted with 100% hexane is identified to be carotenoid pigments **(Figure 2)**.The carotenoid pigments eluted with hexane was collected and stored in vials at -20ºC.

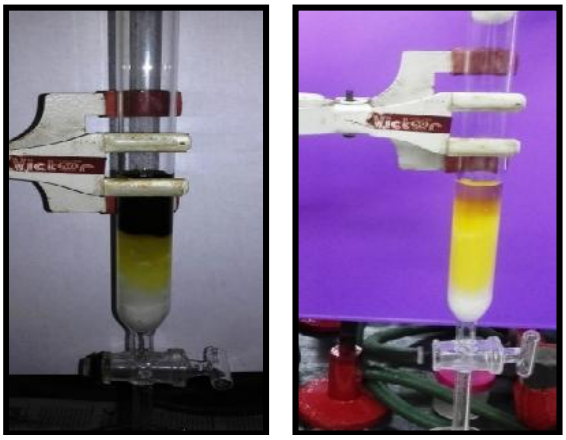


Figure 2: Isolation of Carotenoid pigment

Quantification of Carotenoids

The total carotenoid content was quantified and the results are as follows.

Total carotenoid content in carrot = $0.252x10x10^4$ / 2600x10 = **0.96 µg/g.**

Total carotenoid content in red spinach = $0.231x10x10^4$ / 2600x10 = **0.88 µg/g.**

Total carotenoid content in green spinach = $0.252x10x10^4$ / 2600x10 = **0.96 µg/g.**

Total carotenoid content in beet root = $0.145x10x10^4$ / 2600x10 = **0.56 µg/g.**

Thin Layer Chromatography

The crude extracts and the purified carotenoid pigments and the standard were subjected to thin layer chromatography**.** The standard used was beta carotene. The mobile phase used was hexane and acetone in the ratio 6:4.The respective Rf values for the Vegetables (Carrot, Beet root, Red spinach and Green spinach) were calculated (**Table 1**)**.**

Anticancer Activity of the Extracts - MTT Assay

The cytotoxicity of the crude extracts of Ethanol, Ethyl acetate, Chloroform and the purified carotenoid pigments of each sample was analysed against human breast cancer cell lines, MCF 7 using MTT assay. It is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2 -yl) 2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase in the live cells. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. Cells were treated with 100µg and 150µg of the crude extract and carotenoid extracts **(Figures 3 - 5).**

Figure 3: Cells before treatment of the extracts

Figure 4: Cells after adding the extracts

Figure 5: Cells after adding MTT Reagent

The Ethyl acetate crude extracts of Carrot, Red spinach and Green spinach and the Ethanol crude extracts of Beet root showed increased cytotoxicity when compared to other two solvents. The crude extract and their respective isolated carotenoid pigment showed higher cytotoxicity. Over all **Carrot** and **Green spinach** gave the best results in anticancer activity among the vegetables **(Figures 6 – 7 and Table 2).**

Table 1: Rf values of samples

Rf Values of Crude Extract and Carotenoid				
Sample	**Ethanol crude**	**Ethyl acetate crude**	**Chloroform crude**	**Carotenoide pigment**
Carrot	0.94	0.91	0.94	0.94
Red spinach	0.95	0.95	0.94	0.94
Green spinach	0.95	0.95	0.94	0.94
Beet root	0.92	0.91	0.94	0.92

Table 2 : Anticancer Activity of Vegetables

Sample	**Conc**	**Ethanol**		**Ehtyl acetate**		**Chloroform**		**Carotenoid**	
		Cell Viability %	**Cell Toxicity %**	**Cell Viability %**	**Cell Toxicity %**	**Cell Viability %**	**Cell Toxicity %**	**Cell Viability %**	**Cell Toxicity %**
Carrot	100	71.22	28.78	64.79	35.21	66.83	33.17	62.75	37.25
	150	62.85	37.15	62.44	37.56	62.85	37.15	51.12	48.88
Beet root	100	61.12	38.88	72.75	27.25	68.46	31.54	71.22	28.78
	150	59.28	40.72	71.22	28.78	67.14	32.86	69.28	30.72
Red spinach	100	67.34	32.66	56.93	43.07	67.04	32.96	67.65	32.35
	150	56.22	43.78	52.55	47.45	66.32	33.68	62.44	37.56
Green spinach	100	72.44	27.56	61.42	38.58	75.1	24.9	62.24	37.76
	150	70.4	29.6	59.18	40.82	69.38	30.62	61.32	38.68

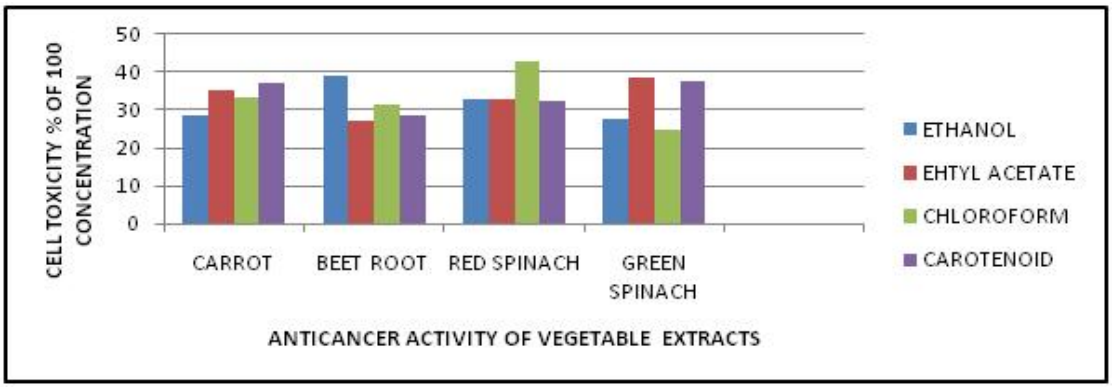


Figure 6: Anticancer activity of Vegetable extracts at 100µl concentration

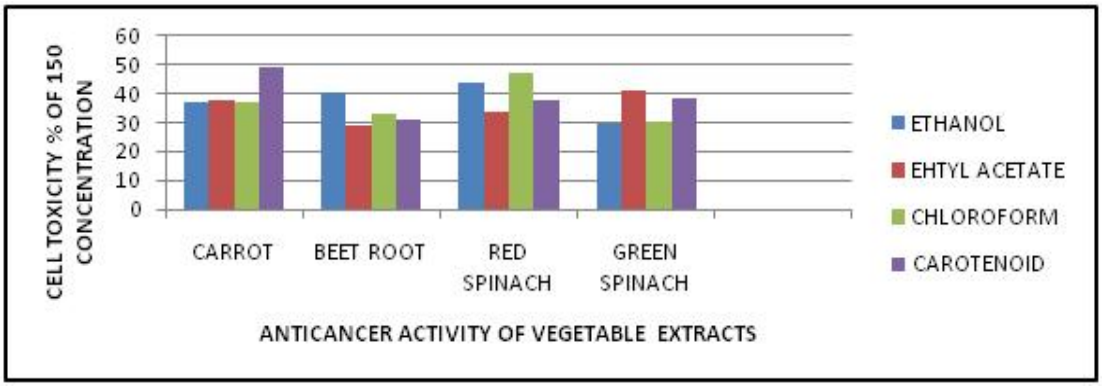


Figure 7: Anticancer activity of Vegetable extracts at 150µl concentration

4. Conclusion

The results of MTT assay on the human breast cancer cell lines, MCF 7 showed dose dependent increase in cytotoxicity of the extracts on the cancer cells. As the concentration of the extracts increases, the cytotoxicity to

the cells increases, suggesting the anticancer activity of the extracts. However, the cytotoxicity percentage was maximum in the isolated carotenoid pigment extracts than the crude extracts of all three solvents. Thus the present study reveals that the Vegetables **Carrot and Green Spinach** to be the best in Anticancer activity and is highly recommended for consumption for prevention of dreadful diseases like cancer.

5. References

[1] Ana P. Tiveron, Priscilla S. Melo, Keityane B. Bergamaschi, Thais M. F. S. Vieira, Marisa A. B. Regitano-d'Arce and Severino M. Alencar (2012) "Antioxidant Activity of Brazilian Vegetables and Its Relation with Phenolic Composition" *International Journal of Molecular Science* 13: 8943-8957.

[2] Bendich A (1989) "Carotenoids and the immune response" *Journal of Nutrition* 119, 112.

[3] Delia-Gabriela Dumbravă, Nicoleta-Gabriela Hădărugă, Daniel I. Hădărugă, Camelia Moldovan, Diana Rab (2010) "Determination by RP-HPLC of β-carotene concentration from orange (*Citrus sinensis* L.) fruits peel extracts" *Journal of Agro alimentary Processes and Technologies* 16(2): 242-246.

[4] Dey P M and Harborne J B (1997) *Plant biochemistry,Third Editon* ,427-429.

[5] Jeszka W.J. (1997) "Food Colorants Chemical and Functional Properties of Food Components" Technomic Publishing Company, Lancaster: 293.

[6] Joanna Fiedor and Kvetoslava Burda (2014) "Potential Role of Carotenoids as Antioxidants in Human Health and Disease" *Nutrients* 6, 466-488.

[7] Onkar P. R. (2013) "Evaluation of phytochemical and pharmacological activity of beetroot extracts (*Beta vulgaris*)" *Biochem & Pharmacol* 2:4.

[8] Rajeswari Satapathy and Paramjyoti Swamy (2012) "Total phenolic, flavonoid content and hepatoprotective potentials of Peltophorum pterocarpum (DC) K Heyne leaf extracts" *Annals of Phytomedicine,* 1(2): 93-96.

[9] Saiful Islam M, Ronok Zahan, Badrul Alam M, Marufa Naznin, Mosaddik M A and Ekramul Haque M (2011) "Pharmacological Study of the *Peltophorum pterocarpum* flower" *International Journal of Pharmaceutical Sciences and Research*, Vol. 2(9): 2309-2313.

[10] Seow-Mun Hue, Amru Nasrulhaq and Chandran Somasundaram (2011) "Comparative Study on the Antioxidant Activity of Leaf Extract and Carotenoids Extract from *Ipomoea batatas* var. Oren (Sweet potato) Leaves" *World Academy of Science, Engineering and Technology*, 58.

[11] Sonia S., Srivastava K., Saxena M. and Mishva R. (2007) "Tomato; A Rich Source of Lycopene and its Role in Human Health – A Review" *Journal of Medicinal and Aromatic Plant Sciences* 29, 81-89.

[12] Velišek J. (1999): Chemie potravin (II). OSSIS, Tabor. "Isolations and formulations of nutrient-rich carotenoids" United States Patent: 6056962.