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Research Article

A STUDY ON THE ANTIOXIDANT ACTIVITY OF CRUDE EXTRACT AND CAROTENOID PIGMENTS FROM VEGETABLES

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ABSTRACT

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. Particularly important are the antioxidant 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. The present study is aimed at studying the Antioxidant activity of Crude Extract and Carotenoid pigments from selected Vegetables of Medicinal Importance.

Keywords: Vegetables, Antioxidant, Phytochemicals, Crude Extract and Carotenoid Pigments.

INTRODUCTION

Natural antioxidants such as vitamin C, tocopherols, flavonoids and other phenolic compounds are known to be present in certain plants[1]**.** Recent studies have shown the importance of vegetables in a healthy diet in preventing degenerative diseases caused by oxidative stress[2]**.** 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 recommended to add a daily intake of it[3] **(Figure 1).**

Figure 1: Dried Vegetable samples

Carrot (*Daucus carota* L.) is the most important crop of *Apiaceae* family. It is a root vegetable that has worldwide distribution. Carrots were first used for medical purposes and gradually used as food. Carotenoids and anthocyanins are the major antioxidant pigments found in carrots. Cultivar differences in carrots rely in the type of pigments present. Carotenoids are the yellow, orange, or red colored phytochemicals found in most yellow and orange fleshed cultivars. The widely used orange carrot is high in *α*- and *β*-carotene and is a rich source of provitamin A. Yellow carrot color is due to lutein which plays an important role in prevention of macular degeneration[4]. Carrots have also a unique combination of three flavonoids: kaempferol, quercetin and luteolin[5]. They are also rich in other phenols, including chlorogenic, caffeic and p-hydroxybenzoic acids along with numerous cinnamic acid derivates[6].

Beetroot is proposed to have beneficial effects in health and disease. Beetroot is a rich source of phytochemical compounds that includes ascorbic acid, carotenoids, phenolic acids and flavonoids[7]. Beetroot is also one of the few vegetables that contain a group of highly bioactive pigments known as betalains[8]. Members of the betalain family are categorised as either betacyanin pigments that are red-violet in colour or betaxanthin pigments that are yellow-orange in colour[9]. A number of investigations have reported betalains to have high antioxidant and anti-inflammatory capabilities *in vitro* and a variety of *in vivo* animal models. This has sparked interest in a possible role for beetroot in clinical pathologies characterised by oxidative stress and chronic inflammation such as liver disease, arthritis and even cancer[10].

Spinach is **loaded with flavonoids** which act as antioxidants, protecting the body from free radicals. Researchers have discovered at least 13 different flavonoid compounds that act as anti-cancer substances. The various nutrients offer much in the way of disease protection. Another of the health benefits of spinach is that this is **a heart-healthy food**. It's an outstanding source of vitamins C and A which are antioxidants that help reduce free radical amounts in the body. The antioxidants work to keep cholesterol from oxidizing. In addition, folate is good for a healthy cardiovascular system, as well as magnesium, a mineral that helps to lower high blood pressure. **Gastrointestinal health** can be guarded by eating more of this food. The beta-carotene and vitamin C work to protect the cells of the body's colon from the harmful effects of free radicals. Also, DNA damage and mutations in colon cells may be prevented by the folate that's present in this green leafy vegetable.

Some conditions that are identified as inflammatory, such as arthritis, osteoporosis, migraine headaches, and asthma, may be helped because of the **anti-inflammatory properties** of some of the nutrients found in spinach. Studies have shown that consumption of green leafy vegetables such as spinach may slow the age-related decline in brain function. Carotenoid found in spinach offers **protection against eye diseases** such as age-related cataracts and macular-degeneration. The mineral iron is particularly important for menstruating women and growing children and adolescents. In comparison to red meat, spinach provides a lot less calories, is fat and cholesterol free, and an **excellent source of iron**. Because iron is a component of hemoglobin, which carries oxygen to all body cells, it's needed for good energy[11].

CAROTENOIDS

Carotenoids are an abundant group of naturally occurring pigments. They occur ubiquitously in all organism conducting photosynthesis. They are found in photosynthetic membranes of phototropic bacteria and cyanobacteria. More than 600 different carotenoids from natural sources have been isolated and characterized [12]. Carotenoids consist of 40 carbon atoms (Tetraterpenes) with conjugated double bonds. They consist of 8 isoprenoid units joined in such a manner that the rearrangement of isoprenoid units is reversed at the centre of the molecule so that the two central methyl groups are in a 1, 6 position and the remaining non terminal methyl groups are in a 1,5 position relationship[13].

Carotenoids besides the anticancerous effect, shows a strong antioxidant character, which plays an important role in the prevention and treatment of cardiovascular, ophthalmological, dermatological diseases and prevents the oxidative damages that are specific to ageing phenomena and also prevents the immunological disorders. Due to carotenoids great sensitivity to light, heat, oxygen and acids, their isolation from different raw materials must be accomplished choosing the optimal work conditions to gum up their degradation[14].

Some carotenoids, such as lycopene, zeaxanthin, lutein, capsanthin, and canthaxanthin are not converted into vitamin A in the body. But again, they are powerful cancer fighters, prevalent in fruits and vegetables. There is abundant evidence that lycopene in particular helps reduce the risk for prostate cancer[15].

The present study is aimed at isolating the 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 their Antioxidant property.

MATERIALS AND METHODOLOGY

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. After 48 hours, the extracts were filtered. The carotenoid pigments were isolated using Column Chromatography and was quantified using the formula

Total carotenoid content (µg/g) = A x V (ml) x 10^4 / $A^{1\%}$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 Antioxidant studies using Reducing Power assay and Phosphomolybdenum methods.

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.

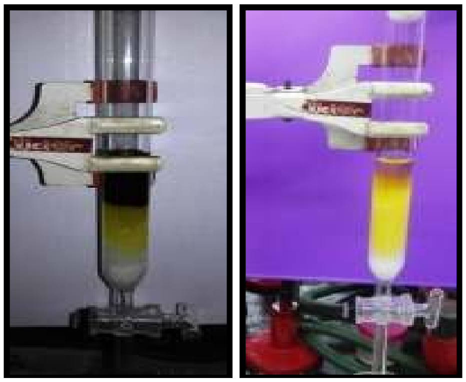

Figure 2: Isolation of Carotenoid pigment

QUANTIFICATION OF CAROTENOIDS

The total carotenoid content quantified 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).**

Table 1 : R_f Values of Crude Extract and Carotenoid

Sample	Ethanol Crude	Ethyl Acetate Crude	Chloroform Crude	Carotenoid 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

ANTIOXIDANT ACTIVITY OF THE EXTRACTS

1. REDUCING POWER ASSAY

The reducing power assay was used to test the reducing capability of the extracts. Their ability to reduce the potassium ferricyanide (Fe3+) complex to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex is determined by measuring the absorbance at 700 nm of sample with standard **(Figure 3).**

The concentration dependent activity was observed in all the crude extracts and carotenoid extracts. The reducing power of the extracts increased with increase in concentration. However, the isolated carotenoid pigments from their respective samples showed higher reducing activity compared to the crude solvent extracts. The Ethyl acetate crude extracts of (Carrot and Green spinach) and the Chloroform crude extracts of Beet root and Red spinach showed increased activity when compared to other two solvents. But their respective isolated carotenoid pigment showed higher activity than the crude. Over all **Carrot** and **Green spinach** gave the best results in Reducing Power assay among the Vegetables **(Figure 4 and Table 2).**

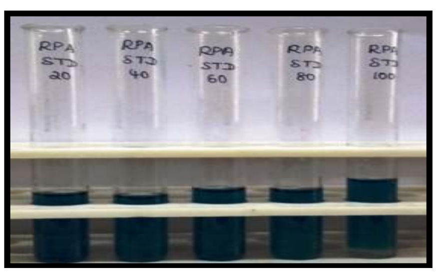

Figure 3: Standard test of Reducing Power assay

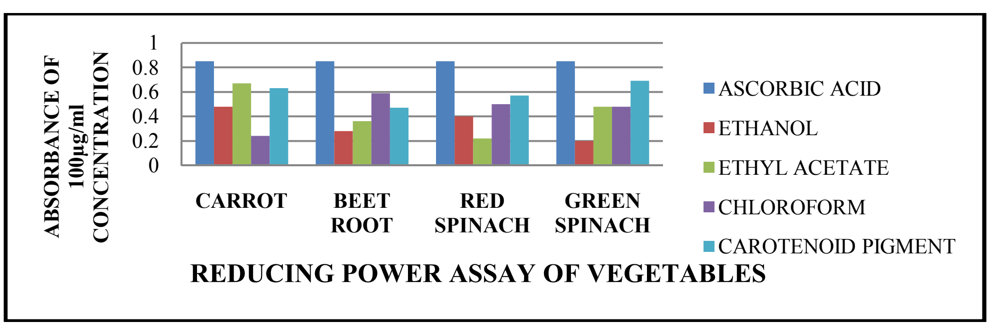

Figure 4: Reducing power activity of Vegetable extracts

Table 2 : Reducing Power Activity of Vegetable Extracts

Sample	Conc (µg/Ml)	Standard Ascorbic Acid Od	Ethanol	Ethyl Acetate	Chloroform	Carotenoid Pigment
Carrot	20	0.17	0.4	0.56	0.16	0.25
	40	0.45	0.42	0.59	0.18	0.32
	60	0.63	0.44	0.61	0.2	0.52
	80	0.80	0.46	0.63	0.22	0.57
	100	0.85	0.48	0.67	0.24	0.63
Beet Root	20	0.17	0.2	0.23	0.51	0.24
	40	0.45	0.22	0.26	0.53	0.29
	60	0.63	0.24	0.29	0.55	0.32
	80	0.80	0.26	0.33	0.57	0.36
	100	0.85	0.28	0.36	0.59	0.47
Red Spinach	20	0.17	0.28	0.14	0.42	0.42
	40	0.45	0.33	0.16	0.44	0.44
	60	0.63	0.36	0.18	0.46	0.45
	80	0.80	0.38	0.2	0.48	0.48
	100	0.85	0.4	0.22	0.5	0.57
Green Spinach	20	0.17	0.12	0.4	0.38	0.29
	40	0.45	0.14	0.42	0.42	0.32
	60	0.63	0.16	0.44	0.44	0.36
	80	0.80	0.18	0.46	0.45	0.47
	100	0.85	0.2	0.48	0.48	0.69

2. TOTAL ANTIOXIDANT ACTIVITY BY PHOSPHOMOLYBDENUM METHOD

The phosphomolybdenum assay was used to determine the antioxidant capacity of the extracts based on the reduction of Mo (VI) – Mo (V) by the antioxidants and subsequent formation of a green phosphate/Mo (V) complex by measuring the absorbance at 695 nm of the sample with standard **(Figure 5).**

The Ethanol crude extracts of Carrot, the Ethyl acetate crude extract of red spinach and the Chloroform crude extract of Beet root and Green spinach showed increased activity when compared to other two solvents. But their respective isolated carotenoid pigment showed higher activity than the crude extract**.** Over all **Carrot** and **Green Spinach** gave the best results in Total antioxidant activity among the Vegetables **(Table 3 and Figure 6).**

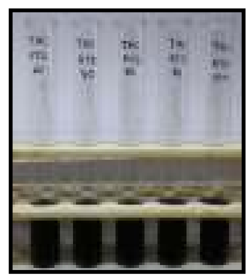

Figure 5: Standard test of Total Antioxidant Activity

Table 3 : Total Antioxidant Activity Of Vegetable Extracts

Sample	Conc (µg/Ml)	Standard Ascorbic Acid Od	Ethanol	Ethyl Acetate	Chloroform	Carotenoid Pigment
Carrot	20	0.16	0.22	0.11	0.26	0.67
	40	0.42	0.27	0.21	0.29	0.69
	60	0.55	0.3	0.23	0.32	0.72
	80	0.74	0.32	0.26	0.36	0.78
	100	0.88	0.35	0.27	0.39	0.88
Beet Root	20	0.16	0.2	0.11	0.23	0.53
	40	0.42	0.23	0.15	0.27	0.55
	60	0.55	0.25	0.19	0.3	0.57
	80	0.74	0.27	0.23	0.36	0.6
	100	0.88	0.29	0.27	0.39	0.61
Red Spinach	20	0.16	0.21	0.25	0.15	0.49
	40	0.42	0.25	0.31	0.19	0.52
	60	0.55	0.28	0.36	0.22	0.54
	80	0.74	0.32	0.39	0.26	0.57
	100	0.88	0.36	0.45	0.31	0.58
Green Spinach	20	0.16	0.18	0.41	0.3	0.54
	40	0.42	0.2	0.47	0.32	0.56
	60	0.55	0.26	0.49	0.36	0.57
	80	0.74	0.3	0.54	0.39	0.59
	100	0.88	0.3	0.59	0.42	0.61

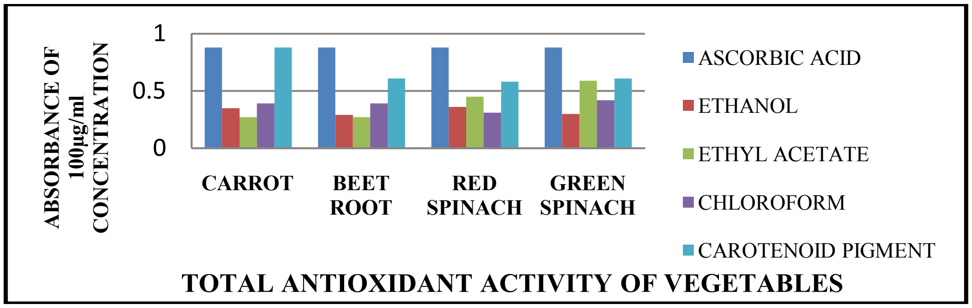

Figure 6: Total Antioxidant capacity by Phosphomolybdenum method

The Reducing power assay and Total antioxidant capacity of the extracts was increased with increase in concentration. However, the isolated carotenoid pigments from their respective samples showed higher reducing activity compared to the crude solvent extracts.

CONCLUSION

The carotenoids were extracted from the Vegetables **(Carrot, Beet root, Red spinach and Green spinach)** by Column Chromatography and subjected to Thin Layer Chromatography. The crude extract and the carotenoid extracts were then analysed for their Antioxidant Activity. The Antioxidant Activity was carried out using Reducing Power Assay and Phosphomolybdenum Method. In both the methodologies done, the carotenoid pigments from the sample **Carrot and Green Spinach** showed highest activity. Thus the present study reveals the Vegetables, **Carrot and Green Spinach** to be the best in Antioxidant activity and is highly recommended for consumption for a healthy living.

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