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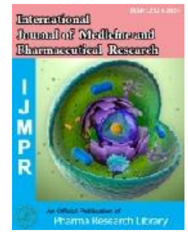

Research Article **Open Access**

Antioxidant Activity of Crude Extract and Carotenoid Pigments from Fruits

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

Citrus fruits, which belong to the family of Rutaceae are one of the main fruit tree crops grown throughout the world. Citrus fruits are well endowed with a variety of phytonutrients. Phytonutrients are vital in both; health promotion and disease prevention. The dietetic and therapeutic properties of all citrus fruits are similar due to their phytonutrient contents. Phytonutrients are mainly natural bioactive compounds from plants with general benefits to human health. The secondary metabolities of plants provide humans with numerous biologically active products, which have been used extensively as food additives, flavours, colors, insecticides, drugs, fragrances and other chemicals. These plant secondary metabolites include several classes such as terpenoids, flavonoids and phenolics compounds having diverse chemical structures and biological activities and exist widely in citrus fruits. The present study is aimed at studying the Antioxidant activity of Crude Extract and Carotenoid pigments from Fruits.

Keywords: Citrus Fruits, Rutaceae, Phytonutrients, Antioxidant activity and Human Health.

A R T I C L E I N F O

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

Citrus plants synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolic hydroxy benzoic and hydroxycinnamic acids, acetophenones, terpenoids, flavonoids, stilbenes and condensed tannins. There are about 40 limonoids in citrus with limonin and nomilin being the principal ones **(D. E.**

Okwi and I. N. Emenike, 2006). These compounds, which occur in high concentration in orange juice (*Citrus sinensis* L.Osbeck) partly, provide the bitter taste in citrus **(J. Zhao, 2007).** Limonoids possess the ability to inhibit tumor formation by stimulating the enzyme glutathione S-transferase (GST).

Orange and lemon oil contains substantial amounts of GST that also possesses anti-cancer activity. Citrus pulp and the albedo (the white of the orange) are rich in glucarates. These substances are being studied extensively for their potentials in preventing breast cancer and lower the risk and symptoms of premenstrual syndrome **(D. E. Okwu, 2008).** Another group of phytochemicals found in citrus are carotenoids. Pink grapefruit have a high content of β-carotene while other citrus fruits such as tangerines and oranges contain high levels of other carotenoids (lutein, zeaxanthin, cryptoxanthin) that have significant anti-oxidant activity. These carotenoids are associated with a lower incidence of age-related macular degeneration, the leading cause of blindness in human after the age sixty five **(J. M. Seddon, A. *et. al.*, 1994).**

All parts of the banana plant have medicinal applications **(Amit and Shailandra, 2006).** Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas **(Brooks, 2008)**. The antibiotic acts against Mycobacteria **(Omojasola and Jilani, 2009)**. A fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants **(Ponnuswamy *et. al*., 2011)**. Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp **(Ratule *et. al*., 2007).** Some of the specific diseases known to be cured by banana are Anaemia as they are believed to be rich in iron to stimulate the production of haemoglobin in the blood and so help in cases of anaemia **(Amit and Shailandra, 2006)**. Banana is extremely high in potassium yet low in salt, making it the perfect food for helping to beat blood pressure **(Debabandya *et. al*., 2010)**. Bananas can also help people trying to give up smoking, as the high levels of Vitamin C, A1, B6, B12 they contain, as well as the potassium and magnesium found in them, help the body to recover from the effects of nicotine withdrawal **(Mokbel, *et. al*., 2005)**. Potassium is a vital mineral, which helps normalize the heartbeat, sends oxygen to the brain and regulates the body's water-balance **(Girish and Satish, 2008).** In recent times, Banana peel has been utilized for various industrial applications including bio-fuel production, bio-sorbents, pulp and paper, cosmetics, energy related activities, organic fertilizer, environmental cleanup and biotechnology related processes **(Morton, 1987; Gunaseelan, 2004; *Bori et.al*., 2007)** It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates **(Singh and Bhat, 2003)**. Pineapples have a serious impact on health, and their health and medicinal benefits include their ability to improve respiratory health, cure coughs and colds, improve digestion, help lose weight, strengthen bones, improve oral health, boost eye health, reduce inflammation, prevent cancer, increase heart health, fight off infections and parasites, improve the immune system and increases circulation. A single serving of pineapple has more than 130% of the daily requirement of vitamin-C for human beings, making it one of the richest and most delicious sources of ascorbic acid. Vitamin C is mainly associated with reducing illness and boosting the immune system by stimulating the activity of white blood cells and acting as an antioxidant to defend against the harmful effects of free radicals. Free radicals are dangerous byproducts of cellular metabolism that can damage various organ systems and disrupt function, as well as cause healthy cells to mutate into cancerous ones. The vitamin C content of pineapples defends against this. High vitamin C content helps to heal wounds and injuries to the body quickly, along with defending against infections and illness. In addition to the antioxidant potential of vitamin C in the battle against cancer, pineapples are also rich in various other antioxidants, including vitamin A, beta carotene, bromelain, various flavonoid compounds, and high levels of manganese, which is an important co-factor of superoxide dismutase, an extremely potent free radical scavenger that has been associated with a number of different cancers. Pineapple has directly been related to preventing cancers of the mouth, throat, and breast **(www.organicfacts.net)**. Pineapple contains a lot of ascorbic acid which is a water-soluble vitamin that has a number of biological functions. Acting as an antioxidant, ascorbic acid important function is to protect Low-density Lipoprotein (LDL) cholesterol from oxidative damage (**Figure 1**).

Figure 1: Dried Vegetable samples

Carotenoids

Citrus carotenoid was demonstrated to have significant reductions in the risk of developing neovascular ARMD as a function of plasma levels of α- carotene, β-carotene, cryptoxanthin, lutein and zeaxanthin. Based on epidemiological data, it can be assumed that diets rich in carotenoid containing fruits are associated with significant decreased risks for a variety of degenerative diseases.

Several epidemiological studies have supported the observation that a high content of blood carotenoids decrease the risk of cataract formation. The ability of carotenoids, to act as antioxidant has been reported **(D. E. Okwu, 2008).** Carotenoids besides the anticancerous effect, showed 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, acids, their isolation from different raw materials must be accomplished choosing the optimal work conditions to gum up their degradation **(Delia -Gabriela Dumbravă *et. al*., 2010).** The present study is aimed at isolating carotenoid pigments from various Fruits such as Orange, Lemon, Pineapple and Banana which are rich in vitamin A, vitamin C and beta carotene and to evaluate and compare its Antioxidant property.

2. Materials and Methods

Orange (Citrus reticulate Blanco)
Lemon (*Citrus limon* (L.)Brum.f.)
Pineapple (*Ananas comosus* (L.)Merr.)
Banana (*Musa acuminate* Colla.)

Preparation of Extracts: The fruits 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 FTIR studies. The antioxidant studies using Reducing Power assay and Phosphomolybdenum methods were carried out.

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 **(Figure2)**.The carotenoid pigments eluted with hexane was collected and stored in vials at -20ºC.

Quantification of Carotenoids

The total carotenoid content quantified are as follows

Total carotenoid content in orange = 0.245x10x10^4 / 2600x10 = **0.94 µg/g.**

Total carotenoid content in lemon = 0.220x10x10^4 / 2600x10 = **0.84 µg/g.**

Total carotenoid content in pineapple = 0.251x10x10^4 / 2600x10 = **0.96 µg/g.**

Total carotenoid content in banana = 0.254x10x10^4 / 2600x10 = **0.97 µg/g.**

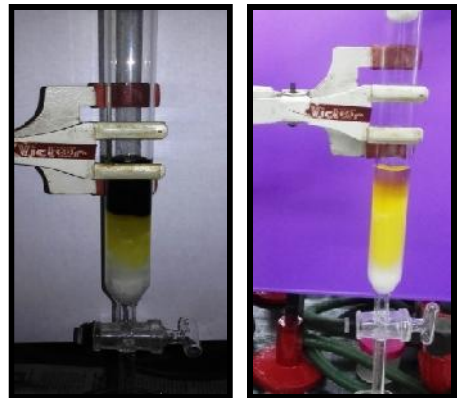

Figure 2: Isolation of Carotenoid pigment

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 fruits (Orange, Lemon, Pineapple and Banana) were calculated (**Table 1).**

Antioxidant Activity of the Extracts

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).**

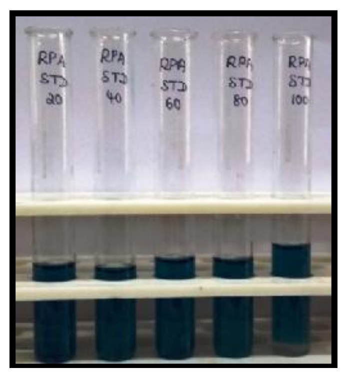

Figure 3: Standard test of Reducing Power assay

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 Ethanol crude extracts of (Pine apple and Banana) and the Ethyl acetate crude extracts of Orange and Lemon showed increased activity when compared to other two solvents. But their respective isolated carotenoid pigment showed higher activity than the crude**.** Over all **Orange** and

Banana gave the best results in Reducing Power assay among the fruits **(Table 2 and Figure 5).**

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 4).**

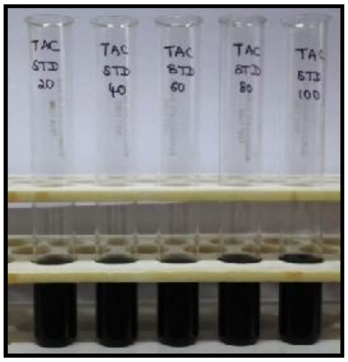

Figure 4: Standard test of Total antioxidant activity

The Ethanol crude extracts of (Orange, Pine apple and Lemon) and the Chloroform crude extract of Banana showed increased activity when compared to other two solvents. But their respective isolated carotenoid pigment showed higher activity than the crude**.** Over all **Orange** and **Banana** gave the best results in Total antioxidant activity among the Fruits **(Figure 6 and Table 3).**

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.

4. Conclusion

The carotenoids were extracted from the fruits (Orange, Lemon, Pineapple and Banana) 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 *Orange and Banana* showed highest activity. Thus the present study reveals the Fruits, Orange and Banana to be the best in Antioxidant activity and is highly recommended for a healthy living.

Table 1: Rf Values of Crude Extract and Carotenoid

Sample	Ethanol crude	Ethyl acetate crude	Chloroform crude	Carotenoide pigment
Orange	0.95	0.94	0.94	0.94
Lemon	0.92	0.92	0.92	0.92
Pineapple	0.92	0.92	0.92	0.92
Banana	0.94	0.91	0.95	0.94

Table 2: Reducing Power Activity of Fruit Extracts

Sample	Conc (µG/ml)	Standard Ascorbic Acid OD	Ethanol	Ethyl acetate	Chloroform	Carotenoid pigment
Orange	20	0.17	0.5	0.48	0.35	0.53
	40	0.45	0.53	0.55	0.41	0.6
	60	0.63	0.55	0.58	0.44	0.63
	80	0.80	0.57	0.62	0.45	0.65
	100	0.85	0.59	0.64	0.5	0.83
Pineapple	20	0.17	0.54	0.3	0.42	0.5
	40	0.45	0.57	0.33	0.44	0.55
	60	0.63	0.61	0.36	0.48	0.58
	80	0.80	0.63	0.39	0.51	0.63
	100	0.85	0.64	0.43	0.53	0.68
Lemon	20	0.17	0.45	0.47	0.16	0.23
	40	0.45	0.47	0.53	0.2	0.24
	60	0.63	0.5	0.57	0.25	0.29
	80	0.80	0.51	0.58	0.27	0.32
	100	0.85	0.56	0.6	0.29	0.36
Banana	20	0.17	0.54	0.42	0.35	0.24
	40	0.45	0.58	0.43	0.37	0.36
	60	0.63	0.61	0.44	0.41	0.45
	80	0.80	0.63	0.45	0.43	0.54
	100	0.85	0.69	0.46	0.45	0.62

Table 3: Total Antioxidant Activity of Fruit Extracts

Sample	Conc (µg/ml)	Standard Ascorbic Acid OD	Ethanol	Ethyl acetate	Chloroform	Carotenoid pigment
Orange	20	0.16	0.63	0.47	0.47	0.65
	40	0.42	0.66	0.49	0.49	0.7
	60	0.55	0.69	0.52	0.54	0.75
	80	0.74	0.74	0.54	0.58	0.79
	100	0.88	0.86	0.57	0.61	0.89
Pineapple	20	0.16	0.57	0.43	0.51	0.47
	40	0.42	0.59	0.46	0.53	0.51
	60	0.55	0.63	0.49	0.56	0.53
	80	0.74	0.65	0.51	0.59	0.56
	100	0.88	0.68	0.53	0.61	0.58
Lemon	20	0.16	0.55	0.43	0.46	0.52
	40	0.42	0.56	0.49	0.48	0.55
	60	0.55	0.57	0.5	0.5	0.57
	80	0.74	0.58	0.51	0.52	0.59
	100	0.88	0.59	0.53	0.56	0.61
Banana	20	0.16	0.51	0.4	0.65	0.55
	40	0.42	0.53	0.44	0.67	0.67
	60	0.55	0.56	0.48	0.69	0.69
	80	0.74	0.59	0.52	0.71	0.74
	100	0.88	0.62	0.58	0.84	0.85

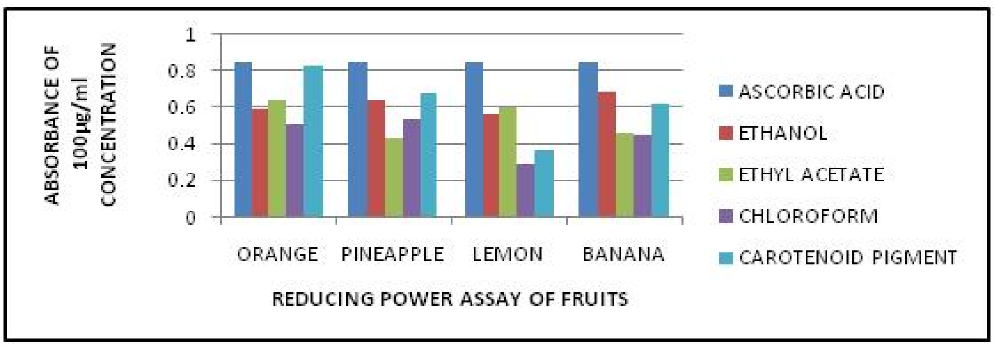

Figure 5: Reducing power activity of Fruits extracts

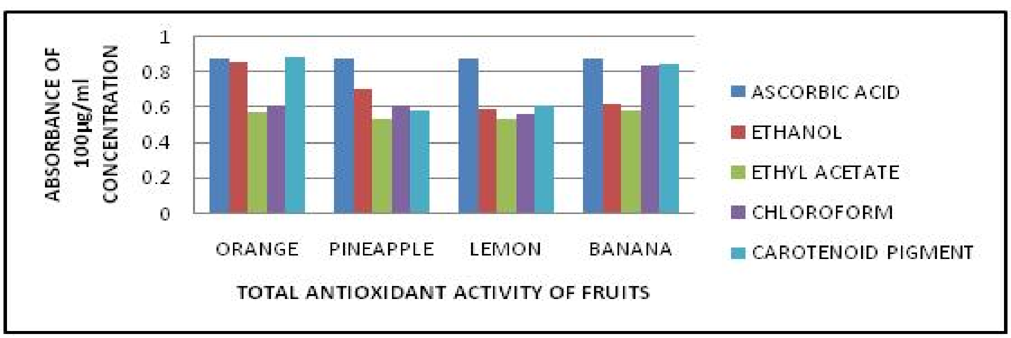

Figure 6: Total Antioxidant capacity by phosphomolybdenum method of Fruits extract.

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