ISSN 2348-375X

Unique Journal of Engineering and Advanced Sciences

Available online: www.ujconline.net

Research Article

A STUDY ON THE ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACT AND CAROTENOID PIGMENTS FROM FRUITS

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Received 26-07-2016; Revised 25-08-2016; Accepted 23-09-2016

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ABSTRACT

The human diet contains important micronutrients namely vitamins C and E, carotenoids and flavonoids which are essential for maintenance of human health. Multiple dietary sources of these compounds are present virtually in all plant material. The nutritional importance of foods is due to the presence of these functional food ingredients and antioxidant nutraceuticals or phytochemicals. Phytochemicals are present in edible fruits and vegetables and when eaten potentially modulate human metabolism in a favourable manner, thereby preventing chronic and degenerative diseases. Increase in fruits and vegetables consumption offers protection against degenerative pathologies such as cancer and atherosclerosis. Thus Citrus fruits are the main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties. It is also scientifically proven that biologically active, non-nutrient compounds found in citrus fruits such as phytochemical antioxidants, soluble and insoluble dietary fibres are known to be helpful in reducing the risk for cancers and many chronic diseases like arthritis, obesity and coronary heart diseases. The present study is aimed at studying the Antimicrobial activity of Crude Extract and Carotenoid pigments of selected Fruits of Medicinal Significance.

Keywords: Human Diet, Phytochemicals, Fruits, Carotenoid Pigments and Antimicrobial Activity.

INTRODUCTION

Ananas comosus (L.) Merrill belonging to the family Bromeliaceae is an important tropical and subtropical plant widely cultivated in the tropical areas of the world. Its fruit is consumed fresh or canned as a commercial product in many countries. Pineapple has also been known for a number of beneficial biological activities such as antioxidative, anti-browning, anti-inflammatory and anti-platelet activities. The enzyme complex of ***A. comosus*** called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation and anti-inflammatory effect[1].

Oranges an excellent source of vitamin C, contains powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases[2]. Consumption of foods rich in vitamin C improves body immunity against infectious agents and scavenging harmful, pro-inflammatory free radicals from the blood. Sweet orange contains a variety of phytochemicals like hesperetin and narigenin. Naringenin has a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator. A high quality orange is one that is mature with good color intensity uniformly distributed over the surface. Such oranges must be firm with a fairly smooth texture and shape that is characteristic of the variety, free from decay, defects and other blemishes. The biological activity and the health effects of citrus flavonoids as antioxidants have been reported[3]. These group of pigments as found in plants and together with anthocyanin play a role in flower and fruit colouration. Also, they are present in dietary fruits and vegetables, and exercise their antioxidant activity in several ways, including the activities of metal chelation[4]. Studies indicate that flavonoids are excellent radical-scavengers of the hydroxyl radical[5], due to their ability to inhibit the hydroxyl radical and donate hydrogen atom[6].

Fruits and vegetables are an important component of a healthy diet. Some fruits like bananas offer great medical benefits. This is partly because bananas aid in the body's retention of calcium, nitrogen, and phosphorus, all of which work to build healthy and regenerated tissues. Bananas can be used to fight intestinal disorders like ulcers. Bananas are one of the few fruits that ulcer patients can safely consume. Bananas neutralize the acidity of gastric juices, thereby reducing ulcer

irritation by coating the lining of the stomach. Not only can bananas relieve painful ulcer systems, and other intestinal disorders, they can also promote healing. Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas. The antibiotic acts against *Mycobacteria*. Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp. The first two elevate blood pressure; serotonin inhibits gastric secretion and stimulates the smooth muscle of the intestines. The fruit is also used as treatment for burns and wounds[7].

Lemon is an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and antibacterial potential [8]. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities[9]. Flavonoids can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation[10]. In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses[11]. The peel of *Citrus* fruits is a rich source of flavonoid glycosides, coumarins, and volatile oils[12]. Many polymethoxylated flavones have several important bioactivities, which are very rare in other plants[13]. In addition the fiber of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being vitamin C (or ascorbic acid), and they certainly prevent and cure vitamin C deficiency-the cause of scurvy[14]. The other health benefits of lime include weight loss, skin care, good digestion, relief from constipation, eye care, and treatment of scurvy, piles, peptic ulcer, respiratory disorders, gout, gums, urinary disorders, etc (**Figure 1**).

Figure 1: Dried Fruit 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 also been reported[15]. Carotenoids are known to suppress the growths of tumors in in vitro (test tube) and in vivo (animal) studies[16]. The various carotenoids such as lycopene, β-carotene, α-carotene, lutein and canthaxanthin can decrease malignant transformation of cells. There has been a positive report on dietary carotenoids improving fertility or reproduction capacity in a number of animals[17].

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, oxidative damages that are specific to ageing phenomena and also prevents 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[18]. 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 Antimicrobial Activity.

MATERIALS AND METHODOLOGY

SAMPLES USED IN THE PRESENT STUDY ARE AS FOLLOWS

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 and the samples were further subjected to Thin Layer Chromatography and Antimicrobial study.

ANTIMICROBIAL ACTIVITY OF THE EXTRACTS

The antimicrobial contents present in the fruit extracts and the carotenoid extracts are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters. Muller Hinton Agar Medium, 24 hour bacterial cultures**,** Sterile Petri plate, Gel puncturing machine and Plant extracts are the materials required.

PREPARATION OF MEDIA

1. NUTRIENT BROTH (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (Hi Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by auto claving at 15lbs pressure (121ºC) for 15 minutes. The broth was cooled to room temperature after sterilizing and then the bacterial cultures were inoculated in them. The cultures were incubated for 24 hours in a shaker at 37°C. These bacterial cultures were used for seeding the petriplates.

2. MULLER HINTON AGAR MEDIUM (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (Hi Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30ml/plate) while still molten.

Petri plates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacteria strain. Wells were made in each of these plates using sterile cork borer. About 100 µl and 75 µl of 100mg/ml concentrations of flower solvent extracts and carotenoid extracts were added into the wells and allowed to diffuse. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

RESULTS AND DISSCUSSIONS

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.

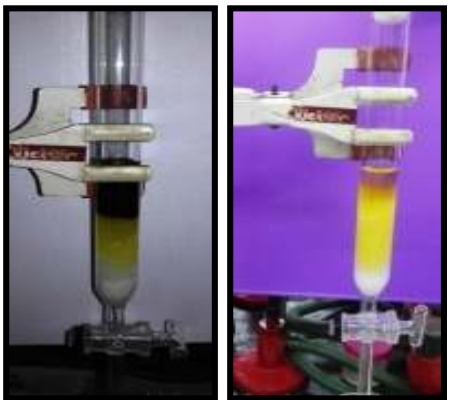

Figure 2: Isolation of Carotenoid pigment

QUANTIFICATION OF CAROTENOIDS

The 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 total carotenoid quantified is 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.**

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).**

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

ANTIMICROBIAL ACTIVITY OF THE EXTRACTS

The antimicrobial activity of the Ethanol, Ethyl acetate and Chloroform crude extracts of the samples includes fruits (Orange, Lemon, Pineapple and Banana) and their respective isolated carotenoid pigments from each sample were studied against organisms namely *Staphylococcus aureus* and *Escherichia coli*. The concentration of each extracts used were 100µg/ml and they were studied using different µl change 100µl and 75µl of each sample extracts. Over all, the extracts of sample of three different solvent showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Table 2).**

Particularly the Ethanolic crude extract of all fruits showed activity against *Staphylococcus aureus* and not against *Escherichia coli* **(Figure 3).** The Ethyl acetate crude extract of all fruits (except lemon) showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Figure 4).** The chloroform crude extracts of fruits (Pine apple and Banana) showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Figure 5).**The Carotenoid pigment extracted from the fruits showed maximum antimicrobial activity against *Staphylococcus aureus* only. The activity was determined by measuring the zone of inhibition in mm.

Table 2 : Zone of Inhibition IN Mm																
Sample	Ethanol				Ethyl Acetate				Chloroform				Carotene Sample			
	Staph		*E .Coli*		*Staph*		*E. Coli*		*Staph*		*E. Coli*		*Staph*		*E. Coli*	
	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl
Orange	10	10	-	-	10	10	10	10	-	-	15	10	15	10	-	-
Lemon	15	10	-	-	-	-	5	-	-	-	10	10	10	15	-	-
Pineapple	15	10	-	-	5	-	5	-	10	-	10	-	10	25	-	-
Banan	10	10	-	-	15	5	20	10	10	-	10	10	15	10	-	-

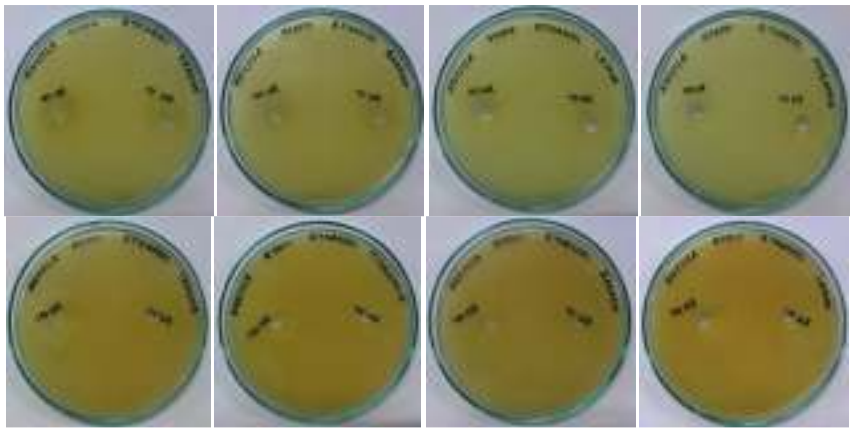

Figure 3: Antimicrobial Activity of Ethanol Crude Extract against organism

Figure 4: Antimicrobial Activity of Ethyl acetate Crude Extract against organism

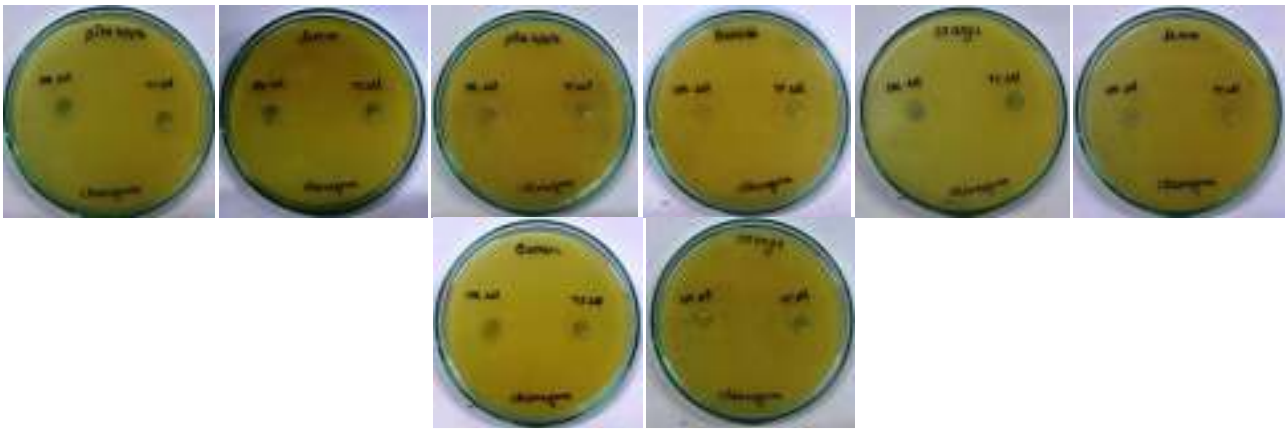

Figure 5: Antimicrobial Activity of Chloroform Crude Extract against organism

CONCLUSION

Thus the present study reveals the Fruits, **Orange and Banana** to be best in antimicrobial activity of the crude extracts and could be attributed to the presence of some metabolic toxins or broad spectrum antibiotic compounds. Thus these extracts could be used as effective antimicrobial compounds in the treatment of diseases.

REFERENCES

1. Manthey JA., Guthrie N., Grohmann K. Biological properties of citrus flavonoids pertaining to cancer and inflammation. Current Medicinal Chemistry, 2001; 8:135–153.
2. Ejaz S., Ejaz A., Matsuda K., Chae WL. Limonoids as cancer chemopreventive agents. Journal of the Science of Food and Agriculture, 2006; 86: 339–345.
3. Tripoli E., La Guardia M., Giammanco, S., Di Majo, D. Giammanco, M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chemistry, 2007; 104: 466–479.
4. Bombardelli E. Morazzoni P. The flavonoids: New perspectives in biological activities and therapeutics. Chimica Oggi, 1993; 25–28.
5. Cillard J., Cillard, P. Compose´s phe´noliques et radicaux libres.*STP Pharma* ,1988; 4:592–596.
6. Di Majo, D., Giammanco, M., La Guardia, M., Tripoli, E., Giammanco, S., Finotti, E. Flavanones in Citrus fruit: Structure antioxidant activity relationships. Food Research International, 2005; 38: 1161–1166.
7. Sampath Kumar KP., Debjit Bhowmik, S. Duraivel, M. Umadevi. Traditional and Medicinal Uses of Banana. Journal of Pharmacognosy and Phytochemistry, 2012; (1): 51.
8. Kawaii ST. Yasuhiko K, Eriko O. Kazunori, Y. Masamichi K. Meisaku ChihiroIto and Hiroshi F. Quantitative study of flavonoids in leaves of *Citrus* plants. J. Agric. Food Chem, 2000; 48: 3865-3871.
9. Burt SA. Essential oils: Their antibacterial properties and potential applications in foods: Av review. Inter. J. Food Microbiol , 2004; 94: 223-253.
10. Duthie G. and A. Crozier. Plant-derived phenolic antioxidants. Curr. Opin. Lipidol, 2000; 11: 43-47.
11. Sohn HY., Son KH, Know CS and Kang SS, Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morusvalba* L., *Morus mongolica* Schneider, *Broussnetiavpapyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis.* Nakai. Phytomedicine , 2004; 11: 666-672.
12. Shahnah SM., Ali S, Ansari H and Bagri P. New sequiterpene derivative from fruit peel of citrus limon (Linn) Burn. F. Sci. Pharm, 2007; 75: 165-170.
13. Ahmad MM., Salim-ur-Rehman Z, Iqbal-Anjum FM and Sultan JI. Genetic Variability to Essential Oil Composition in Four Citrus Fruit Species. Pak. J. Bot, 2006; 38(2): 319-324.
14. Aronson, J.K. Nature Publishing Group. Retrieved from: http://medicine.nature.com, 2001.
15. Okwu DE. Citrus fruits: a rich source of phytochemicals and their roles in human health. International Journal of Chemistry and Science, 2008; 6(2): 451-471.
16. Sonia S., Srivastava K., Saxena M. and Mishva R. Tomato; A Rich Source of Lycopene and its Role in Human Health – A Review. Journal of Medicinal and Aromatic Plant Sciences, 2007; 29: 81-89.
17. Bendich A. Carotenoids and the immune response. Journal of Nutrition, 1989; 119(1): 112-115.
18. Delia-Gabriela Dumbravva, Nicoleta-Gabriela Hadaruga, Daniel IH, Camelia Moldovan, Diana Rab. Determination by RP-HPLC of β-carotene concentration from orange (*Citrus sinensis* L.) fruits peel extracts. Journal of Agro alimentary Processes and Technologies, 2010; 16(2): 242-246.

Source of support: Nil, Conflict of interest: None Declared