



Estimation of Antioxidant Levels in Peels of Pomegranate, Banana, Orange, Lemon, Sweet Lime

Avneet Kaur, Yashita Jain, Kashish Mittal, Armin Kaur and Nidhi Mittal*

Department of Biotechnology, GGSDS College, Sector-32 C, Chandigarh 160 030, India

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ABSTRACT In the present study antioxidant levels were evaluated in pomegranate (*Punicagranatum*), lemon (*Citrus limon*), sweet lime (*Citrus limetta*), banana (*Musa acuminata*) and orange (*Citrus sinensis*) peels. Level of the various phytochemicals like phenolics, tannins, sugars, proteins, ascorbic acid and flavonoids, present in fruit peels were determined. The antioxidant activity of fruit peels was done by ferric-reducing antioxidant power method (FRAP). The carbohydrate levels present in sweet lime peels (34.68 ± 1.06 mg/ml) was significantly higher than in pomegranate (31.1 ± 1.41 mg/ml) and banana (17.18 ± 0.44 mg/ml) peels ($p \leq 0.05$). Significantly high levels of ascorbic acid were observed in sweet lime peels (14.39 ± 0.78 mg/ml) as compared to lemon (3.05 ± 1.19 mg/ml), orange (3.87 ± 0.77 mg/ml), and pomegranate (3.44 ± 0.16 mg/ml) peels ($p \leq 0.05$). In the present study significantly higher antioxidant and phenolic levels was reported in pomegranate peels (60.93 mg TAE/g) as compared to other investigated fruit peels ($p \leq 0.05$). Highest flavonoid levels were observed in orange peels ($56.6 \text{ mg} \pm 1.48 \text{ AAE/g}$) in comparison to other fruit peels.

INTRODUCTION

In India, rise in middle class and fast economic growth has not only led to increased consumption of fruits as processed food such as juices, jams, jelly powders, fruit bars but also generation of fruit wastes (principally peels and seeds) in large amounts. However, the disposal of fruit waste materials has come out to be a costly affair leading to negative impact on the environment (O'Shea et al. 2012). In India, about 18 percent of the fruit and vegetables production worth Rs. 44,000 crores are going waste annually. The level of fruits and vegetable processing is only 4 percent due to highly decentralized fruit processing industry (Ghosh et al. 2016).

The fruit waste, especially fruit peels are source of sugars, proteins, organic acids, dietary fibre and bioactive compounds such as phenolic compounds which act as source of natural antioxidants (Medina et al. 2017). The peel and

seed fractions of some fruits such as pomegranate, orange, banana have higher antioxidant activity than the pulp fractions due to presence of polyphenols, such as flavonoids, tannins, and catechins (Singh et al. 2001). The multiple biological activities of polyphenols and their potential use in the prevention of many human diseases such as cardiovascular disease, obesity, diabetes, among others have been widely recognized (Dzialis et al. 2016).

India is contributing 27 percent of the world banana production. Banana peel which contributes to 40 percent of the total weight of fresh banana is an underutilized source of phenolic compounds (Vu et al. 2018). Banana peels are a rich source of starch (3%), crude protein (6-9%), crude fat (3.8-11%), total dietary fibre (43.2-49.7%), and polyunsaturated fatty acids and are used for food flavoring and production of wine and ethanol production (Mohapatra et al. 2010). Banana peels are considered as a good source of antioxidants for functional foods against cancer and heart disease (Someya et al. 2002).

Citrus is the largest fruit crop worldwide, with approximately 100 million metric tons produced annually (FAO 2006). It was reported that peels of orange represent between 50 to 65 percent of the weight which are discarded as by-product (Hegazy and Ibrahim 2012). The natural flavor of citrus fruit peels (orange, lemon, sweet lime, lime, etc.) has several benefits in food like in candies, yogurt and cosmetic industries (Adeel et al. 2014). Citrus peels contain pectin which is

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*Address for correspondence:

Dr Nidhi Mittal

Assistant Professor

Dept. of Biochemistry,

GGSDS College, Sector-32C,

Chandigarh 160030, India

E-mail: avbawa@yahoo.co.in

used as a gelling agent particularly in jams and jellies, and used also in medicines and sweets (Suliman et al. 2013).

Pomegranate peel represents about 40-50 percent of the total fruit weight (Gullon et al. 2016). Al-Rawahi et al. (2014) has reported around 61 different polyphenols in pomegranate peels which include 12 hydroxycinnamic acids, 14 hydrolysable tannins, 9 hydroxybenzoic acids, 5 hydroxybutanedioic acids, 11 hydroxycyclohexanecarboxylic acids and 8 hydroxyphenyls. The hydrolysable tannins present in pomegranate peel include hexahydroxydiphenic acid (HHDP) and its derivatives, ellagic acid and punicalin, and punicalagin which have strong antioxidative properties.

In Ayurvedic medicine, the peels of pomegranate and orange are known as suppressors of inflammation which may contribute to chemotherapeutic and chemopreventive utility against cancer (Sharma et al. 2017). The peels are a source of bioactive compounds such as phenolics, flavonoids, ellagitannins, proanthocyanidin compounds, minerals, and complex polysaccharides which contributes to its antioxidant activity (Deo and Shakhale 2018). Very few studies have been carried to do comparative analysis of phenolic, flavonoid and antioxidant levels in various peels from fruits commonly consumed by Indian population.

Objective

The objective of the present study was to evaluate the antioxidant capacity as well as levels of phytochemicals like phenolics, tannins, sugars, proteins, ascorbic acid and flavonoids in fruit peels of pomegranate (*Punicagranatum*), lemon (*Citrus limon*), sweet lime (*Citrus limetta*), banana (*Musa acuminata*) and orange (*Citrus sinensis*).

METHODOLOGY

Processing of Fruit Peels

Fruit peels of pomegranate, lemon, sweet lime, banana and orange were collected separately from local market and dried in hot air oven at 70°C for 3-5 days. The dried fruit peels were powered and stored at room temperature. One

gram of dried fruit peels was weighted into a beaker and 100 ml of boiling distilled water was added. After brewing for 5 min, the blend was removed and the extract was cooled down and all tests were done in triplicate.

Estimation of Protein, Total Sugar Content

The protein concentration of the fruit peel extract was done by the Lowry protein assay method (Lowry et al. 1951). The sugar content was estimated at 620nm using glucose as a standard (Dubois et al. 1951).

Estimation of Ascorbic Acid levels

The 2,4- dinitrophenylhydrazine (DNP) method was used to determine the ascorbic acid levels in respective samples. The procedure is an adaptation of an analytical method in which reduced ascorbic acid is oxidised and dehydroascorbic acid, followed by coupling with 2,4-dinitrophenylhydrazine under controlled conditions gives red coloured osazones (Roe and Keuther 1943). To 0.6 ml of fruit peel extract, distilled water was added to make volume up to 3 ml. Then, 1 ml of 2,4-DNP was added to each tube followed by incubation at 37°C for 3 hours. 7 ml of 80 percent H₂SO₄ was then added to each tube in order to completely dissolve the red osazones crystals. Total amount of ascorbic acid was calculated using standard curve of ascorbic acid at wavelength of 540 nm.

Determination of Tannin Content

The tannins were determined by Folin-Ciocalteu method (Naima et al. 2012). About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 percent Na₂CO₃ solution and volume was adjusted to 10 ml with distilled water. The mixture were mixed well and kept at room temperature for 30 min. A set of reference standard solutions of ascorbic acid (20, 40, 60, 80 and 100 ig/ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of AAE /g of extract.

Estimation of Total Phenol Content (TPC)

The total phenol content (TPC) was determined by spectrophotometer using tannic acid as standard with some modifications (Kaur et al. 2015). 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The TPC was expressed as mg tannic acid equivalents (TAE)/ g. The concentration of polyphenols in samples was derived from a standard curve of tannic acid ranging from 10 to 100 µg/ml.

Determination of Total Flavonoid Content

Total flavonoid content was measured according to the modified aluminium chloride colorimetric assay (Kaur et al. 2015). The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 percent sodium nitrite was added and after 5 minutes, 0.3 ml of 10 percent aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and volume was raised to 10 ml with distilled water. A set of reference standard solutions of ascorbic acid (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg AAE/g of extract.

Determination of Antioxidant Power by Using Modified Ferric Ion Reducing Antioxidant Power Assay (FRAP)

The total antioxidant capacity was determined spectrophotometry, using ascorbic acid as standard according to the modified FRAP assay (Kaur et al. 2015). 0.1 ml of extract was taken and to it 0.9 ml of 75 percent ethanol, 5 ml of distilled water, 1.5 ml of 1M HCl, 1.5 ml 1 percent potassium ferricyanide, 0.5 ml of 1 percent SDS and 0.5 ml of 0.2 percent of ferric chloride was added. This mixture was boiled in water

bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 750 nm to measure the reducing power of the extract. The antioxidants in samples were derived from a standard curve of ascorbic acid ranging from 10 to 100 µg/ml. The total antioxidant power was expressed as mg ascorbic acid equivalent (AAE)/ g.

Statistical Analysis

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one way ANOVA ($p \leq 0.05$) using GraphPad Prism software.

RESULTS AND DISCUSSION

The various fruit studied in the present study contained significant amount of carbohydrates. However, high amount of carbohydrates was found in sweet lime, banana and pomegranate as compared to lemon and orange, as reported in literature (Maniyan et al. 2015; Aquino et al. 2016) (Table 1). The carbohydrates present in sweet lime peels (34.68 ± 1.06 mg/ml) were significantly higher than pomegranate (31.1 ± 1.41 mg/ml) ($p \leq 0.05$) and banana (17.18 ± 0.44 mg/ml) peels ($p \leq 0.05$) (Table 1). The significant high amount of carbohydrates in citrus peel especially sweet lime could be due to galacturonan containing pectic substances such as cellulose, glucans, arabinan, and xylan (Terpstra et al. 2002) and polysaccharides such as polygalacturonic acids and cellulose glucosan (Torrado et al. 2011). Dafny-Yalin et al. (2010) have reported the presence of mannitol, glucose and fructose in pomegranate. The sugars present in pomegranate also possess antioxidative properties due to the formation of complexes between pomegranate polyphenols and sugars (Rozenberg et al. 2006; Aviram et al. 2008).

Ascorbic acid which functions as a chemical reducing agent or antioxidant was found in high amount in fruit. Significantly high levels of ascorbic acid were found in sweet lime peels (14.39 ± 0.78 mg/ml) as compared to lemon (3.05 ± 1.19 mg/ml), orange (3.87 ± 0.77 mg/ml) and pomegranate (3.44 ± 0.16 mg/ml) peels ($p \leq 0.05$) (Table 1). Since ascorbic acid is not synthesized de novo, peels could be a very good source of ascorbic

Table 1: Carbohydrate, ascorbic acid, tannins, protein, antioxidant, flavonoid and phenolic levels in the considered samples of fruit peels (banana peels, orange peels, lemon peels, sweet lime peels, pomegranate peels)

Samples	Carbohydrates (mg/ml)	Protein (mg/ml)	Ascorbic acid(mg/ml)	Tannins (AAE/g)	Antioxidants (AAE/g)	Flavonoids (AAE/g)	Phenolics (TAE/g)
Banana(Musa acuminata)	17.18 ± 0.44	9.70 ± 0.05	1.32 ± 0.15	17.82 ± 1.40	11.62 ± 0.01	41.2 ± 3.53	10.53 ± 2.82
Orange(Citrus sinensis)	10.62 ± 0.88	12.72 ± 0.99	3.87 ± 0.77	19.81 ± 1.41	16.13 ± 0.28	56.6 ± 1.48	55.3 ± 8.48
Lemon(Citrus limon)	4.1 ± 0.38	17.85 ± 0.07	3.05 ± 1.19	5.09 ± 1.19	14.03 ± 0.42	53.6 ± 2.82	9.86 ± 2.26
Pomegranate (Punica granatum)	31.1 ± 1.41	38.80 ± 2.10	3.44 ± 0.16	38.05 ± 7.38	22.92 ± 0.43	52.16 ± 0.70	60.93 ± 2.82
Sweet lime (Citrus limetta)	34.68 ± 1.06	15.06 ± 0.19	14.39 ± 0.78	29.11 ± 3.21	15.63 ± 0.70	53.66 ± 4.24	24.53 ± 1.13

acid needed for collagen hydroxylation, carnitine biosynthesis, and norepinephrine formation in human body (Levine et al. 1999).

In the present study, significantly high amount of tannins were observed in pomegranate peels (38.05 ± 7.38 mg AAE /g) ($p \leq 0.05$) as compared to orange (19.81 ± 1.41 mg AAE /g), banana (17.82 ± 1.40 mg AAE /g) and lemon (5.09 ± 1.19 mg AAE /g) peels ($p \leq 0.05$) as shown in Table 1. Al-Rawahi et al. (2014) have reported that the tannins and flavonoids present in the pomegranate are illogie acid, gallic acids, punicalin, and punicalagin and anthocyanidins such as cyanidin, pelargonidin, and delphinidin (Noda et al. 2002). In this study, the tannins levels in banana were significantly higher ($p \leq 0.05$) than in lemon and orange peels as reported by Kondo et al. (2016). Min et al. (2003) reported that moderate levels of tannins present in fruit can protect protein degradation in the rumen and increase amino acid absorption in the small intestine.

Various bioactive phenolic compounds are present in fruit which include gallic acid and hydroxycinnamic acid and its derivatives, including ferulic, sinapic, p-coumaric, chlorogenic and caffeic acid. The phenolic compound were found in all the fruit studied that is pomegranate peels (60.93 ± 2.82 mg TAE/g) followed by orange (55.3 ± 8.41 mg TAE/g), sweet lime (24.53 ± 1.13 mg TAE/g), banana (10.53 ± 2.89 mg TAE/g), lemon (9.86 ± 2.62 mg TAE/g) peels as reported in literature (Singh and Immanuel 2014; Ghasemi et al. 2009) (Table 1). Phenol acids such as ferulic and sinapic acids were found highest in citrus fruits (Kondo et al. 2005). The main phenolic compounds in banana were chrysin, quercetin and catchin (Aboul-Enein et al. 2016). The phenolics in fruit could be useful for anthelmintic effect, which requires a strong protein binding capacity in the intestine (Hoste et al. 2006). Mertens-talcott et al. (2016) reported that fruit peel phenolics especially those present in pomegranate are available during the digestion in quite a high amount (29%) due to absorbability of ellagitannin (EA) from a pomegranate extract high in EA content and its *ex vivo* antioxidant effects. Kondo et al. (2016) reported that phenolics in fruit such as banana peel may bind proteins moderately in the rumen, but they may exhibit lower binding activity in post-rumen, as similar to green tea.

The highest amount of flavonoids were found in pomegranate (52.16 ± 0.70 mg AAE/g) followed by citrus (53.6 ± 2.82 mg AAE/g to 56.6 ± 1.82 mg AAE/g) and least was found in banana peels (41.2 ± 3.53 mg AAE/g) (Table 1). The flavonoids present in pomegranate are kaempferol, luteolin, and quercetin (Van Elswijk et al. 2004). The citrus fruit peel flavonoids comprises of flavanones, flavones and anthocyanins. The citrus flavonoids include a class of glycosides, namely, hesperidin and naringin and another class of O-methylated aglycones of flavones such as nobiletin and tangeretin, which are relatively two common polymethoxylated flavones (PMFs) (Li et al. 2006). The flavonoids present in orange can be used as natural sweeteners as glycosylated flavanones can be easily converted into the corresponding dihydrochalcones.

In the present study, for determination of antioxidant levels, aqueous extraction with boiling was done as it increases the solubility of phenols and enhances the breakdown of high molecular weight phenolics into free form leading to efficient extraction of polyphenols (Sathishkumar et al. 2009). However for the use of fruit peels as antioxidant in food products, various factors such as form of antioxidant (powder or solution), method and time of incorporation are particularly important for the dispersion of antioxidant and ultimately stabilization of the product. Singh and Immanuel (2014) have reported that pomegranate, orange and lemon peels can be used safely at the level of 1-2 percent of antioxidant extract. The range of antioxidants present in fruit peels was 11.62 ± 0.01 mg AAE/g to 22.92 ± 0.43 mg AAE/g (Table 1). Significantly higher levels of antioxidants were found in pomegranate (22.92 ± 0.43 mg AAE/g) as compared to other fruit peels which could be due to the presence of total tannins and purified constituents (for example, ellagic acid and punicalagins) as reported earlier by Maniyan et al. (2015). The antioxidant activity of orange peels is due to glycosylated flavanones such as hesperidin and naringin (M'hiri et al. 2017). The antioxidants present in orange peels exhibit antidiabetic effect by potentiating the antioxidant defense system and suppressing pro-inflammatory (Vinayagam and Xu 2015). The peels of banana contain various antioxidant compounds such as gallic catechin (Kanazawa and Sakakibara 2000),

dopamine (Someya et al. 2002), ferulic acid and caffeic acid (Vu et al. 2018).

CONCLUSION

Thus, fruit peels are rich source of sugars, protein, phenolics and flavonoids which have a wide range of action which includes anti-tumoral, anti-viral, anti-bacterial, cardio protective and anti-mutagenic activities. These fruit peels can be used as novel, natural and economic sources of antioxidants, which can be used in the prevention of diseases caused by free radicals. The present study revealed that among all the five fruit peels extracts studied, significant higher antioxidant levels was reported in pomegranate peels as compared to other fruit peels making it "a pharmacy unto itself" as said in Ayurvedic medicine.

RECOMMENDATIONS

Fruit peels are a cheap source of bioactive compounds especially antioxidants. However, further studies are needed for identification of the active constituents that can help in the development of food products and beverages with enhanced shelf life and antioxidant properties for use in food industry.

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