Research Article

Comparative study of antioxidative properties of Jhelum valley fruits

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Abstract

The significance of antioxidant are well known in biosphere, recently become more evident with circular economy and recycling methodologies. Owing to this property of fruits and vegetables, are effective in prevention of chronic diseases. The use of fruits as antioxidants is of global importance because of their accessibility and affordability when compared with conventional medicine. In the current study we performed antioxidant activities in selected fruit samples using the 2,2-diphenyl-picrylhydrazyl (DPPH) antioxidant analysis. DPPH the free radical removal method provides first approach to assess the potential antioxidant compound, extract, or other biological sources. A simplified method, in which the extract of potential compound is mixed with the solution of DPPH reagent and the absorbance, is noted after a specified period. Results of DPPH antioxidant analysis of Jhelum Valley, Azad Jammu & Kashmir (AJK) showed that all the three samples i.e. Apple, Peach and Pomegranate are good scavengers of free radicals. The overall trend of decrease in radical scavenging activity is Pomegranate > Apple > Peach. The fruits samples also have phenolic and flavonoids properties. Experimental findings demonstrate that almost all the fresh fruits are rich in antioxidant with and added ability of free radical scavenging.

Keywords: Anti-oxidative properties; Apple; DPPH; Fruits; Moisture; Peach; Phenolic content; Pomegranate

Introduction

Free radicals are the molecules having unpaired electron those are highly reactive biologically and chemically. Free radicals are necessarily produced in biological systems by exposure to different physiochemical or pathological state endogenously [1-5]. The attacks of free radicals, in living cells to continuous damage, known as oxidative stress results in various retrogressive disorders, like mutagenesis, fibrosis, atherosclerosis, neurodegenerative carcinogenesis, arthritis, cardiovascular diseases and ageing [6, 7].

Antioxidant compounds, act as free radical scavenger, which neutralize free radicals by mediated oxidative chain either with one of the known phenomena's such as initiation, termination and propagation. Variety of such antioxidants are naturally formed and present in number of organic products, e.g. fruits, vegetables, etc. [1-8]. Natural antioxidants have great importance that may protect human cells against the dangerous effects of free radicals produced [9]. In human body, these antioxidants are first defense line against free radical damage or abnormal tissue growth. Regular

consumption of various vegetables and fruits has been recognized as reducing the risk of degenerative diseases [10, 11].

One of the objectives of this study was to estimate total moisture contents in three commonly consumed fruits i.e. Pomegranate (*Punica granatum*), Peach (*Prunus persica*) and Apple (*Malus pumila*); the source samples of this study were collected from common species of Jhelum valley of Azad Jammu and Kashmir, Pakistan. Another objective was to determine antioxidant activity by estimating total phenolic contents and total

flavonoids contents in these fruit samples [1-3].

Materials and Methods

The fruit samples were collected from Chikar, District Jhelum valley of AJK, Pakistan. About 50 g of each fruit sample such as pomegranate, peach, and apple were taken in weighed petri dishes separately. Samples were kept in microwave oven at 50°C for 10 days for getting average constant weight and its moisture content is calculated using below formula:

% moisture contents

$$= \frac{\text{Wt. of fruits samples} - \text{Wt. of oven dried fruits samples}}{\text{Wt. of fruits samples}} \times 100$$

Evaluation of antioxidant potential of fruits samples, DPPH-radical scavenging potential were conducted for total phenolic content and total flavonoid content.

Preparation of methanolic extract of fruits samples

The 50g pulp of fruits samples were dried at 50°C for 10 days, until constant weight. Every fruit sample was ground to powder form using pistol and mortar. Then 2g of each fine dried fruit powder was soaked in 100ml of 80% methanol and stirring at room temperature (25°C) for about 24 hours and filtered using Whatman paper. After evaporation, the residues were diluted to 20 ml using 80% methanol [12].

DPPH scavenging activity

In order to determine the DPPH scavenging activity, methanolic extract of 2.5ml of every sample were mixed with 1ml of 3mM DPPH-methanol solution. The mixture was incubated at room temperature in dark for 30 min. Finally, the mixture's absorbance was measured at 517nm in comparison to a blank (without DPPH). Assay performed in triplicate [13]. For the quantification of the antioxidant activity, standard ascorbic acid calibration curve ($R^2 = 0.9899$) was created by running solutions in the concentration range of 0.02-0.1 mg/ml; by using spectrophotometer. The total phenolic

contents were expressed as ascorbic acid equivalent (AAE) mg/ g of said dry weight (DW) [13].

Total phenolic contents (TPC)

Contents of total phenolic compound were evaluated using *Folin-Ciocalteu* solution [14]. In a test tube, 1 ml of methanolic extract from each sample was added to 1 ml of *Folin-Ciocalteu* solution. After 3 minutes, 1 mL sodium carbonate solution (20%) was added to the mixture. For 30 minutes, the mixture was left to sit at room temperature in a dark area. At 725nm, absorbance was measured against a blank reagent. The experiment was carried out three times. A standard Gallic acid calibration curve, developed by running solutions in the concentration range of 0.05–0.25 mg/ml ($R^2 = 0.9987$), was used to calculate the TPC quantity. Gallic acid equivalent (GAE) mg/g were used to measure overall antioxidant activity [14].

Total flavonoids contents

Total flavonoid contents were determined by taking 0.5 ml of sample extracts. Each fruit sample's methanolic extract was diluted in 2 ml distilled water and mixed with 0.5 ml of 10% AlCl_3 (aluminum chloride) and the same volume of sodium acetate (1M). Finally, 2 mL distilled water was added and allowed to sit for 30

minutes. The absorbance of a 4ml methanolic extract without aluminum chloride was then measured at 415 nm against a blank sample. The experiment was carried out three times [15]. Quantitative data were calculated using a standard calibration curve with Rutin hydrate concentrations ranging from 0.1 to 0.5 mg/ml [1-3, 15]. Rutin hydrate equivalent in milligrams per gram of dried fruit sample weight was used to calculate total phenolic content.

Results and Discussion

Total moisture contents of fruits samples

Following (Table 1) shows that the total moisture contents in fruits samples, i.e. Apple, Peach and Pomegranate. Peach has highest moisture contents and the Pomegranate has least percent moisture contents. The overall trend of decrease in the percent moisture content is Peach > Apple > Pomegranate

Table 1. Results of total moisture contents in fruits samples

Sr. No.	Fruits Samples	Percent moisture contents
1	Apple (<i>Malus pumila</i>)	83.7%
2	Peach (<i>Prunus persica</i>)	84.96%
3	Pomegranate (<i>Punica granatum</i>)	79.8%

Results of antioxidant activity with DPPH Assay

All the experimental work performed on three fruits samples has given the following results of their antioxidant potential with

DPPH assay. A standard curve for ascorbic acid has been drawn (Fig. 1) to compare the radical scavenging activity of fruits samples using DPPH radical scavenging activity.

Figure 1. Standard curve of Ascorbic acid for DPPH radical scavenging Assay

Antioxidant potential of fruits samples using DPPH radical scavenging assay

The stable free radical DPPH has a deep violet color and a maximum absorption wavelength of 515 nm (Fig. 2). Due to the abstraction of hydrogen atoms from

antioxidants, the violet color of DPPH is decreased to a pale-yellow tint in this assay. Antioxidant potential of fruits samples were determined by using DPPH radical scavenging activity shown by (Table 2).

Figure 2. Graphical representation of % DPPH scavenging activity of fruits samples

Table 2. Antioxidant potential of fruits samples

Sr. No.	Fruit samples	mg/g of ascorbic acid equivalent
1	Apple (<i>Malus pumila</i>)	4.21
2	Peach (<i>Prunus persica</i>)	2.81
3	Pomegranate (<i>Punica granatum</i>)	6.42

The (Table 2) show that the pomegranate has highest concentration of antioxidant potential and peach has least concentration.

And the percentage radical scavenging activity of (%RSA) was quantified using the formula:

$$\%RSA = \frac{[(A \text{ control} - A \text{ sample})]}{A \text{ control}} \times 100$$

Where A control represents absorbance of control reaction for A fruit sample.

In the literature, there are numerous researches about analysis of antioxidant activity in pomegranate apple and peach, which give slightly different results (Table 3). The obtained results have been indicated that antioxidant activity of the Egyptian pomegranate extract, which is similar to the results reported by Soulemana and Ibrahim [16]. The Methanol extracts of Pomegranate (Ripened Pomegranates from Khorasan province in Iran) seed had the highest antioxidant activity 0.15mg/g were reported by Basiri *et al.* [17]. Manzoor *et al.* [18] measured the DPPH radical scavenging activity of 80 percent methanolic extracts of pulp from different cultivars of apple from Pakistan, finding that Red Delicious had the highest (51.1 percent) and Kashmiri Amri (apple type)

had the lowest (42.9 percent) scavenging ability respectively, which is almost similar to present result. Drogoudi *et al.* reported that the greatest antioxidant activity was found in apple varieties at commercial maturity (11.9 to 3.7 mg /g DW) from an experimental orchard at Pomology Institute in Naoussa, Greece [19]. Manzoor *et al.* [20] found that DPPH radical scavenging activity of 80 percent methanolic extracts from several kinds of peach from Swat (Khyber Pakhtunkhwa) and Quetta (Baluchistan) had the highest (49.1%) and lowest (43.4%) scavenging capacity, respectively. Mokrani *et al.* [21] reported the antioxidant activity of peach cultivars (from Algeria) by DPPH method using tolrox equivalent as standard, 16.0 to 48.9 mg/g TE which is higher than current result. The variation in the results due to the modification in assays or using different

standards or varieties from different regions. Sahin *et al.* [22] reported that Pomegranate exhibited the highest

antioxidant capacity, followed by apple and peach. And the values were of the same order as reported in this publication.

Table 3. Comparison of antioxidant activity by DPPH

Sr. No.	Fruit sample	mg /g	% scavenging activity	Reference 1	Reference 2
1	Pomegranate	6.42	71.%	70% [16]	0.15 [17]
2	Apple	4.21	57.9%	51% [18]	3.7 [19]
3	Peach	2.81	35.6%	49.1% [20]	16.0 [21]

Results of phenolic contents

Total phenolic contents were determined using Gallic acid standard method. The standard curve is drawn (Fig. 3) by using Gallic acid which is naturally occurring

organic compound with phenolic properties. The standard Gallic acid curve is drawn to compare total phenolic contents of each fruits sample.

Figure 3. Standard curve of Gallic acid as standard for total phenolic contents

Total phenolic contents of fruits samples using Folin-ciocalteu

Total phenolic contents of every fruit sample was measured by using *folin-ciocalteu* (Fig. 4). The (Table 4) show that the Apple has least concentration of phenolic properties and apple has highest concentration of phenolic properties. The overall trend of decrease in the activity is: Peach > Pomegranate > Apple

Same order of decreasing TPC such as peach 2.6 mg/g GAE > Pomegranate 2.4 mg/g GAE > apple 1.9 mg/g GAE were reported by Sahin *et al.* [22]. Bassiri-Jahrom *et al.* [23] recently presented a comprehensive profile of total polyphenolic

and flavonoid contents of pomegranate cultivators throughout the world. These studies suggest that the PG 1-5 cultivators of Egypt are quite rich in polyphenols (86.7-123.7 mg/g GAE), while the Turkish cultivars are quite low in phenolic content (0.117-0.177 mg/g GAE as compared to our sample (1.94 mg/g GAE). The same trend is seen in the pomegranate juices of these cultivators (Table 5). However, the results are comparable to the juice of Italian cultivars (2.06mg/ml GAE), while the Chinese varieties show slightly higher values (4-7 mg/g GAE) [23]. Henriquez *et al.* reported that the phenolic content in pulp extract of apple varieties (from Molina,

Curicó, Maule Region, Chile, during the summer 2007) by *Folin-ciocalteau* using standard Gallic acid. The phenolic contents ranging from 1.5 to 2.3 mg/g GAE, which is similar to present result [24]. The amount of total phenolic content in three peach

varieties (of Pakistan) ranged from 711.7–881.3 mg GAE/100 g (mean = 791mg/100g which is equal to 7.91mg/g) of DW for pulp extracts were recorded by Manzoor *et al.* [20].

Figure 4. Total phenolic contents with reference to standard Gallic acid

Table 4. Total phenolic contents of fruits samples

Sr. No.	Fruits Samples	mg/g of Gallic acid equivalent
1	Apple (<i>Malus pumila</i>)	1.91
2	Peach (<i>Prunus persica</i>)	2.25
3	Pomegranate (<i>Punica granatum</i>)	1.94

Table 5. Comparison of total phenolic contents mg/g GAE

Sr.	Fruit sample	Current result	Reference 1	Reference 2	Reference
1	Pomegranate	1.94	2.4 [20]	0.117 [21]	8.67 [14]
2	Apple	1.91	1.9 [20]	1.5 [22]	2.475 [23]
3	Peach	2.24	2.6 [20]	7.91 [18]	0.4 [24]

Phenolic contents in the Egyptian pomegranate cultivars ranged from 8.67–12.37 mg/g GAE were reported by Soulemana and Ibrahim [16] which was slightly higher than current results. Leontowicz *et al.* [25] reported that total phenolic contents of apple 2475mg/kg GAE (which is equal to 2.475mg/g GAE) which is almost similar to current result. Campbell *et al.* [26] were presented a phenolic contents of New York peach varieties ranged from 40–95 mg/100g GAE (0.4–0.95mg/g GAE) which is also similar to current result. The variations in the

results due to the modification in assays or different varieties from different regions or the different stages of the ripening of fruits etc.

Result of total flavonoids contents in fruits samples

Total flavonoids contents of fruits samples were measured by standard curve of Rutin hydrate (RE) as standard. A standard curve of Rutin hydrate is drawn (Fig. 5) to compare the flavonoids properties of fruits samples. The (Table 6) shows that pomegranate has highest concentration of flavonoids contents. The overall trend of

decrease in activity is: Pomegranate > Apple > Peach (Fig. 6). Sahin *et al.* [22] reported that Peach exhibited the highest flavonoid contents as compared to pomegranate and apple. Elfalleh *et al.* [27] identified the flavonoid contents in Gabsi pomegranate variety (Southern Tunisia) was 6.79mg/g RE which is similar to current result. Li *et al.* [28] reported that the flavonoid content of pomegranate Chinese variety was 17.2mg/g RE which is higher than the current result. Total flavonoid contents in apple varieties (from Pakistan) varied from 7.11-9.99mg/g Catechin equivalent (CE) were reported by Manzoor *et al.* [20]. Leontowicz *et al.* [25] reported

that the total flavonoid contents of apple 14mg/100g CE (0.14mg/g CE) which is almost similar to current result. The mean value of the amount of total flavonoid content in the three different varieties of peach (Pakistan) 349mg/100g CE (3.49mg/g CE) was reported by Manzoor *et al.* [20]. Zhang *et al.* [29] were reported that the flavonoid contents in the pulp extract of 33 peach cultivars from China varied from 4.35- 35.51 μg RE/g (0.00435-0.03551mg/g RE) which is slightly lower than current result (Table 7). The variation in the results may be due to the peach varieties from different region.

Figure 5. Standard curve of Rutin hydrate for flavonoids contents

Table 6. Total flavonoids contents of fruits samples

Sr. No.	Fruits Samples	mg/g of Rutin hydrate equivalent
01	Apple (<i>Malus pumila</i>)	3.48
02	Peach (<i>Prunus persica</i>)	7.53
03	Pomegranate (<i>Punica granatum</i>)	4.30

Figure 6. Graphical representation of flavonoids contents in fruits samples with reference to Rutin hydrate as standard

Table 7. Comparison of total antioxidant activity

Sr. No.	Fruit sample	Current result mg /g RE or CE	Reference 1	Reference 2
1	Pomegranate	7.53	6.79 [27]	17.2 [28]
2	Apple	4.3	7.11 [18]	0.14 [25]
3	Peach	3.48	3.49 [20]	0.035 [29]

Correlation among antioxidant potential, phenolic contents and flavonoids contents in fruits samples: The result show that all the three fruits samples are good scavenger of free radicals. A comparative observation among antioxidant potential, total phenolic contents and total flavonoids contents of fruits samples shows that these fruits have highest concentration of flavonoids contents. The overall trend of decrease in these activities:

Flavonoids contents > Phenolic contents > Antioxidant potential

Good relationships between antioxidant activities and total phenolic content were identified by Mokrani *et al.* [21] indicating that the phenolic compounds contained in these fruit peach cultivars are substantial contributors to their total antioxidant potential.

Conclusion

Many fruits have medicinal characteristics, and their inclusion in our diet on a regular basis can help us avoid oxidative stress illnesses. Antioxidants are found in fruits in the form of phenolic compounds, ascorbic acid, carotenoids, and tocopherols, among other things. It is well recognized that phenolic chemicals, such as flavonoids from plants, are responsible for the majority of radical scavenging. The results of antioxidant activity by DPPH radical scavenging supported that all the three samples studied contain significant amount of antioxidant compounds. All the samples showed antioxidant potential with variation in free radical scavenging, phenolic and flavonoid activity. Apples are low in fat and rich sources of dietary fibers, minerals and vitamins. Therefore, intake of apples in human diet is advised in cholesterol

controlling and weight reducing programs. The minor differences in nutritional composition may be due to the difference in fruit variety, origin and growth condition. Peach contain the antioxidant elements which reduce the risk of prostate cancer, amount of vitamin C and glutathione, powerful antioxidant which strengthens immune system and it improve overall blood flow by acting as vasodilator. Pomegranate provides antioxidants vitamins A, C and E. All this make pomegranate very suitable for skin and retinal conditions. Antioxidants are helpful in the treatment of a variety of diseases because of their ability to scavenge free radicals. Antioxidant activity was determined in term of ability of antioxidants in the fruits to inhibit oxidation. Therefore, in short, a balanced diet contain fruits and vegetables provide desired antioxidants for good health.

Authors' contributions

Conceived and designed the experiments: I Karim & AA Mughal, Performed the experiments: I Karim, Analyzed the data: I Karim, M Khalid & AA Mughal, Contributed materials/ analysis/ tools: I Karim, M Khalid & AA Mughal, Wrote the paper: I Karim & M Khalid.

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