Comparison of Ascorbic Acid, Total Phenolic Content and Antioxidant Activities of Fresh Juices of Six Fruits Grown in Oman

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Abstract

Background: Regular consumption of fruits and their juices play a beneficial role in the prevention of many diseases owing to the presence of dietary antioxidants such as ascorbic acid, flavonoids and polyphenols.

Aim: The aim of the study was to analyze and estimate total phenolics, ascorbic acid content and to investigate free radical scavenging activity of six locally grown and commonly eaten fruits in Oman.

Methods: The fruits were thoroughly washed and mechanically squeezed to obtain the juices. The fruit juices after filtration were analyzed for total acidity, ascorbic acid, total phenolic content and in-vitro antioxidant activity by alkalimetry, iodimetric (volumetric), Folin Ciocalteu reagent (colorimetric) and 1,1, diphenyl picrylhyrazyl (DPPH) assay methods respectively.

Key findings: Lime juice was found to be the most acidic while grapefruit juice had the maximum ascorbic acid content. Total phenolic content of the juices were observed in the following order of decreasing levels: pomegranate>grapefruit>lemon>lime>sweet melon>watermelon. The result of in-vitro antioxidant activity of all juices, barring watermelon and sweet melon, were quite significant, but the highest scavenging activity of DPPH radicals was exhibited by pomegranate juice closely followed by grapefruit and lemon juices respectively.

Conclusion: The results of this study indicated that these fruits are important to the consumer’s health and a potential source of dietary antioxidants due to their high phenolic and ascorbic acid contents.

Keywords: Antioxidant activity; Ascorbic acid; DPPH; Total phenolics

Introduction

Oxidative stress is believed to be involved in causation of more than hundred chronic diseases such as cancer, diabetes, cardiovascular and degenerative diseases etc. [1]. One of the possible ways to fight these diseases is to reinforce our body’s antioxidant defense system. Antioxidants possess the ability to scavenge the free radicals and protect the body from damages caused by the oxidative stress [2]. In order to strengthen our immunity and to maintain good health, we must supply our body with the natural antioxidants present in exogenous sources such as vegetables, fruits, cereals, grains, beverages and hot drinks etc through the balanced diet [3].

Fruits are important part of a healthy diet and are rich in vitamins, fibres, minerals, micro nutrients and phenolic compounds [4]. Fruits have very high antioxidant value in comparison to vegetables and cereals [5]. Therefore, regular consumption of fruits and their juices play a beneficial role in the prevention and progression of many chronic diseases owing to the presence of dietary antioxidants such as ascorbic acid, flavonoids and polyphenols [6-8]. Fruits are also routinely consumed as fresh juices due to their suitability for ingestion which supply a relevant part of intake of health promoting polyphenolic phytochemicals [9].

Fruits and vegetables constitute the important part of Omani diet. Fruits are the fourth largest source of food nutrition and in quantitative terms it is more than the average daily consumption of Fish and meat together in Oman [10]. Fruits such as lime, lemon, grapefruit (citrus), melons (water and sweet) and pomegranate are the local commercial agricultural produce of Oman. Citrus fruits are known for their high ascorbic acid content, a natural antioxidant that protects the organism from oxidative stress [11] but in addition to ascorbate, there are other phytochemicals present such as phenolics and anthocyanins that contribute more to the antioxidant capacity than ascorbate [12].
**Preparation of fruit juices**

The fruits were washed thoroughly with water to remove dust and the juices were mechanically squeezed from the fresh fruit. The juices were filtered using a Buchner funnel; the clear fruit juice was collected in clean containers and kept in refrigerator at 4°C till further use.

**Determination of pH**

The pH of the fresh juices was measured by using a digital pH meter (RI060P, Thermo Electron Corporation, Singapore) immediately after extraction at room temperature.

**Determination of total acidity**

Neutralization titration method was used to determine the total acidity of the juices. Previously standardized 0.1N Sodium hydroxide was used as a titrant to quantify total acidity of fresh juices using phenolphthalein indicator. The titration was repeated three times and total acidity is expressed as g/L of the juice in terms of citric acid equivalent [13].

Total acidity (mg/L) = Normality of juice × equivalent weight of citric acid

**Determination of ascorbic acid content**

The concentration of ascorbic acid in fruit juice samples was determined by direct iodine redox titration (iodimetry) method by titrating a known aliquot of juice against the standardized iodine solution [14]. The results are expressed as g/100 mL of the ascorbic acid in fresh juice.

**Determination of total phenolic content**

Folin-Ciocalteu reagent method was used to estimate the total phenolic content in the fruit juices as per the reported method [15]. Watermelon acid was used as a reference standard (20-100 µg/mL) for plotting the calibration curve. The total phenolic content was calculated as mean± SD (n = 3) from the standard plot of watermelon acid and expressed as mg/mL watermelon acid equivalent (GAE) of fruit juice.

**Determination of antioxidant activity**

The free radical scavenging activity of different concentration of fruit juices (10 µL to 100 µL/mL) was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the previously reported procedure [16]. Ascorbic acid was used as a positive control for comparison purpose. The percent (%) radical scavenging activity of the tested fruit juice samples and standard ascorbic acid was calculated by using the following formula:

\[
\text{% free radical scavenging activity} = \left( \frac{A_c - A_x}{A_c} \right) \times 100
\]

Where

- \( A_c \) = Absorbance of control (DPPH) alone at 517 nm and
- \( A_x \) = Absorbance of fruit juices and DPPH combination

**Results**

Preliminary phytochemical analysis of fruit juices revealed the presence of major classes of secondary metabolites but phenolic compounds and carbohydrates were commonly present in all six juices. The pH of fresh juices ranged from 2.79 to 6.77 and citrus fruit juices were found to be more acidic than pomegranate and melon. The pH of citrus fruits was found to be in the order *Citrus aurantium* > *Citrus limon* > *Citrus paradise* while the sweet melon was least acid and its pH was close to neutral (Table 1). The total acidity of fruit juices was determined in terms of citric acid and the results are presented in Table 1. The total acidity of lime juice was found to be the highest (12.48 g/L) and it was approximately double than lemon (6.49 g/L) and four times of grapefruit juice (3.2 g/L). The least acidic content was found in sweet melon (0.48 g/L).

Ascorbic acid is the most important water soluble antioxidant and its concentration in juices estimated by the volumetric method was found to be in the range of 0.0587 to 0.709 g/100 mL (grapefruit> lime> lemon> sweet melon> watermelon> pomegranate) (Table 1). The total phenolic content of the various juices is expressed in terms of watermelon acid equivalent and are presented in Table 1. The total phenolic contents were calculated using the following linear regression equation obtained from the standard plot of watermelon acid;

\[
A=0.001x + 0.021, \quad r^2=0.991
\]

Where

- \( A \) is absorbance and \( x \) is amount of watermelon acid in µg.

Pomegranate juice was found to contain the highest amount of phenolic compounds closely followed by grapefruit juice. The total phenolic content was least in the watermelon juice.

The antioxidant activity of all the six fruit juices and standard ascorbic acid was investigated by DPPH free radical scavenging method and is expressed as % inhibition. All the juices showed a dose dependent scavenging activity of DPPH comparable to standard antioxidant. It was interesting to note that pomegranate juice exhibited significant antioxidant activity at concentrations of 50 and 100 µL/mL and was comparable to standard antioxidant though it contains the least amount of ascorbic acid (Figure 1). Thus this activity could be attributed to its high phenolic content.

**Discussion**

Fruits or their fresh juices are becoming a part of a healthy diet and are purely consumed for refreshment and nutritional purposes. These are excellent sources of vitamins, minerals, fibres and biologically active phytochemicals. Most of the fresh juices contain varying amount of water soluble vitamin C (ascorbic acid) which is the main nutritional component of these juices. However, humans obtain this essential body nutrient and natural antioxidant from food and supplements because they are unable to synthesize this *in - vivo* [17]. In addition to ascorbic acid, many other organic acids and phenolic compounds are also present in fruits that exhibit potential biological activities such as antioxidant, anti-inflammatory, antimicrobial, anticancer etc. [18, 19]. The antioxidant activity of phenolic compounds is mainly due to their reducing properties which allow them to act as metal chelators, absorb and neutralize free radicals [20]. Many previous studies have

| Fruit juice | pH | Total acidity, g/L | Ascorbic acid content, g/100 mL | Total phenolic content mg GAE/mL |
|-------------|----|-------------------|-------------------------------|-------------------------------|
| Lime        | 2.79 | 12.48 ± 0.22 | 0.645 ± 0.022 | 0.468 ± 0.019 |
| Lemon       | 2.81 | 6.49 ± 0.01 | 0.616 ± 0.042 | 0.569 ± 0.031 |
| Grapefruit  | 4.40 | 3.2 ± 0.06 | 0.709 ± 0.014 | 0.668 ± 0.071 |
| Watermelon  | 5.94 | 0.94 ± 0.07 | 0.0763 ± 0.012 | 0.246 ± 0.020 |
| Sweet melon | 6.77 | 0.48 ± 0.35 | 0.270 ± 0.013 | 0.306 ± 0.021 |
| Pomegranate | 4.51 | 5.16 ± 0.27 | 0.0587 ± 0.012 | 0.776 ± 0.092 |

Values are expressed as mean ± SD, n=3; GAE= watermelon acid equivalent

Table 1: pH, total acidity, ascorbic acid and total phenolic content in the six fruit juices.
clearly demonstrated a correlation between phenolic content level and antioxidant activity in various food stuffs including fruits [21]. However, the concentration of ascorbic acid and phenolic compounds in fruits may vary depending upon the geographical conditions and environmental factors. Therefore, we determined and compared the ascorbic acid, total phenolic content and antioxidant activity in juices of six fruits that are grown in various regions of the Sultanate of Oman.

Results of our study have shown a good correlation between total phenolic content and DPPH scavenging activity of the fruit juices (r² = 0.81) (Figure 2) which in accordance with the previously published reports [22]. Pomegranate juice was found to contain the highest content of total phenol (0.776 mg/mL) and it also exhibited the highest antioxidant activity (93.6%). All the citrus fruits exhibited high antioxidant activities comparable to standard antioxidant in published reports [22]. Pomegranate juice was found to contain the highest antioxidant activity (93.6%). All the citrus fruits exhibited high antioxidant activities comparable to standard antioxidant in published reports [22].

Figure 1: DPPH scavenging activity (% inhibition) of various fruit juices and Ascorbic acid at 517 nm.

Figure 2: Correlation between total phenolic content and antioxidant activity of fruit juices.

Conclusion

The juices upon quantification by colorimetric method were found to be rich in phenolic compounds (tannins and flavonoids) and therefore exhibited very good free radical scavenging activity. Based on the results, it can be concluded that these fruit juices could be used as a natural source of antioxidants and their regular consumption could provide health benefits to humans by shielding against oxidative stress.

Acknowledgment

The authors are grateful to Dean, Associate Dean and Pharmacy Head of Oman Medical College for providing infrastructure and necessary research facilities.

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