Evaluation of antioxidant properties of methanolic extracts from different fractions of quince (Cydonia oblonga Miller).

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Abstract

Quince is the rich source of metabolites with interesting biological properties. In vitro antioxidant capability of methanolic extract of the different fractions of quince (peel, pulp seeds and mucilage) was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Total phenolics, Reducing power, FRAP value and H2O2 scavenging activities. The results obtained for DPPH (43.20%-69.40%), TPC (41.23 mg GAE/100 g-97.51 mg GAE/100 g) RP (31.90%-74.40%) FRAP (0.63 µM-1.16 µM) and H2O2 activity (14.2%-34.2%) revealed a significant difference in antioxidant properties among fractions. The results indicate that peel and seeds which otherwise are wasted, are effective in scavenging free radicals and can serve as potential antioxidants.

Keywords: Quince, Antioxidants, Polyphenols, Phytochemicals, Health benefits

Introduction

Quince (Cydonia oblonga Miller) is the fruit of a deciduous tree of Rosaceae family [1], which is cultivated mostly for its nutritional and medicinal value in South Africa, Middle East and central Europe. The quince fruit is considered to be the rich source of functional and nutritional compounds. Several studies have showed that quince fruit is a good and low-cost natural source of phenolic acids and flavonoids [2-4], which are potent antioxidants. The fruit is supposed to contain comparatively large amount of cell- wall polysaccharide [5, 6] which makes it as a potential source of dietary fibers and pectin. Among dietary antioxidants, phenolic compounds, secondary metabolites from plants, are the most abundant natural antioxidants [7]. Quince fruits were characterized by the presence of mono and dicafeoylequinic acids (3-O-cafeoylequinic, 4-O-cafeoylequinic, 5-Ocafeoylequinic, and 3, 5-O-dicafeoylequinic acids). Quercetin-3-O-galactoside, quercetin-3-O-rutinoside, kaempferol-3-O-glucoside and kaempferol-3-O-rutinoside have been found in pulp. Additionally, peels were characterized by the presence of several quercetin and kaempferol derivatives acylated with p-coumaric acid. Quince seed had a distinct phenolic profile, composed by several C-glycosil flavones (lucenin-2, vicenin-2, stellarin-2, isoschaftoside, schaftoside, 6-C-pentosyl-8-C-glucosyl chrysoeriol and 6-C-glucosyl-8-C-pentosyl chrysoeriol [8-11].

All parts of the plant are considered to be good source of large number of bioactive substances like vitamin (A, C, E, riboflavin, folic acid and K), carotenoids (α, β, δ-carotene and lycopene), and flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glucoside compounds [12]. The fruit has attracted attention of several researchers because of its health promoting properties [13]. The medicinal value of these plants is related to their
phytochemical components which produce definite physiological actions on human body [14]. Dietary fibre is regarded as an important functional component of fruit for human health because it has been associated with a lower risk of several gastrointestinal diseases [15]. Moreover, the pectic polysaccharides have also been reported to have a number of pharmacological actions, such as hypoglycemic, cholesterol-decreasing, and antiulcerative activity [16]. Phenolics and pectins present in fruits and their extracts are potential antiulcerative factors; hence it is possible that the quince may prevent ulcers. Phenolics are able to act as antioxidants in a number of ways. These antioxidants act as reducing agents, hydrogen donors, free radicals scavengers, and singlet oxygen quenchers and, therefore, as cell savours [17]. Phenolics, because of their strong antioxidant capacity along with anti-inflammatory, anticarcinogenic and antiallergic effects, are regarded as one of the functional compounds that contribute to the health-improving effects of the fruit and its derivatives [18-20]. The putative protective effects of antioxidants against the deleterious oxidative-induced injuries have received increasing attention in recent times, especially within biological, medical, nutritional, and agrochemical areas. The fruits were used for the treatment of dysentery. In addition, the seeds of the plant are used as a pharyngeal demulcent and emulsifying agent in the preparation of hair-fixing lotions [21, 22]. Because of their richness in antioxidants, functional compounds and their various described health benefits, this study was carried out to compare antioxidant potential of various fractions of quince so that these can be utilized for nutraceutical purpose in future.

Materials and Methods

The fruits of fresh nature and high quality were brought from the local market to the department of the Food Science and Technology University of Kashmir Srinagar. Fruits of uniform maturity were selected, washed and pulp, peel seeds and mucilage were carefully separated. Seeds were separated from the fresh pulps.

Chemicals and reagents

All chemicals, solvents and reagents used for assessing antioxidant screening were purchased from Sigma-Aldrich Chemie (Buchs, Switzerland) and HiMedia, India.

Sample preparation

After the separation of the fruit into different fractions the fresh pulp was chopped and homogenized for 10 s and the non-edible portion (seed, peel and mucilage) was triturated using blender. Extracts of quince fractions for antioxidant activity and total phenolic measured in methanol were prepared adapting method of [23], with some modifications. Two grams of each sample were mixed with 8 mL methanol and homogenized using homogenizer. The homogenate were incubated at 4 °C for 12 h and then centrifuged at 15,000 rpm using cooling centrifuge (Eppendorf, 5810R). The supernatants were recovered and stored at -18 °C for analysis.

Antioxidant activity of different fractions of quince

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity

The method of [24] as modified by [25] was adopted for measuring the DPPH radical scavenging ability of methanolic extracts obtained from various fractins of quince. The methanolic extracts of all samples were dissolved in 1.0 mL of a 0.1mM DPPH methanol solution at room temperature. After 30 min of incubation the absorption at 515 nm was measured by spectrophotometer (Hitachi, U-2900). The DPPH free radical scavenging activity was calculated as follows:

Radical scavenging activity (SA) = (A0 – A/A0) X 100

A0 is the absorbance of the control
A is the absorbance of the sample

Determination of total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu assay [26] with minor modifications. 100 μL of test sample, diluted appropriately with water, or gallic acid standard were mixed with 500 μL Folin–Ciocalteu reagent and 2 mL of 10% sodium carbonate solution and distilled water was added to reach a final volume of 10 mL. The mixture was stirred and kept for 30 min at room temperature in the dark. The absorbance was measured at 725 nm with spectrophotometer (Hitachi U-2900). The results were expressed in gallic acid equivalents (GAE; mg/100 g fresh mass) using a gallic acid (0–0.1 mg/mL) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

Determination of the reducing power

The determination of reducing power was carried out as per the method described by [27] with the minor modifications. Methanolic extracts were dissolved in 2.5 mL of the 0.2 M phosphate buffer solution (pH 6.6). After this 2.5 mL of the 10% potassium ferricyanide were added to it. Mixture was incubated at 50 °C for 20 minute of time period. To this solution was added 2.5 mL of 10% (TCA) trichloro acetic acid (w/v) and the centrifugation was carried out at 3000 rpm for 10 minutes. The upper layer of 2.5 mL was collected and to which was added 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl3 solution. The absorbance was measured at 700
nm by the spectrophotometer (Hitachi U-2900). The increased absorbance of the reaction mixture indicated increased reducing power. The percentage reduction was calculated as by the following

\[ \text{Reduction (\%age)} = \frac{(A_{\text{test}} - A_{\text{blank}})}{A_{\text{blank}}} \times 100 \]

Where \( A_{\text{test}} \) = absorbance of the sample
\( A_{\text{blank}} \) = absorbance of the control

**Determination of the FRAP (Ferric reducing antioxidant power)**

FRAP (ferric reducing antioxidant power) was determined by the method of [28] as modified by [29]. FRAP reagent was prepared as a mixture of 10 mL of 300 mM acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ in 40 mM hydrochloric solution, and 1 mL of 20 mM FeCl\(_3\) solution. The FRAP reagent, once prepared, was immediately incubated for 10 min at 37 °C, then 3 mL of the reagent was added to 0.1 mL of the extracts of different fractions of quince. The reaction mixture was incubated for 4 min at room temperature and the absorbance at 593 nm was measured using a spectrophotometer (Hitachi U-2900). The value was expressed in µM FRAP/g fresh weight sample.

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 min) / (Change in absorbance of standard from 0 to 4 min) x FRAP value of standard.

**Estimation of \( \text{H}_2\text{O}_2 \) scavenging activity**

The \( \text{H}_2\text{O}_2 \) scavenging activity of antioxidants from the various fractions of the quince was determined according to the method of [30], modified by [31]. Based on UV spectrophotometer \( \text{H}_2\text{O}_2 \) has optimum absorbance at 230 nm, depending on its concentration. Solution of \( \text{H}_2\text{O}_2 \) (3.5 mM) and extracts were prepared in phosphate buffer (pH 7.4) followed by the determination of concentration by spectrophotometer at 230 nm. After that, extracts (antioxidants) at different concentrations were added to \( \text{H}_2\text{O}_2 \) solution, separately. The absorbance of \( \text{H}_2\text{O}_2 \) at 230 nm was determined at specific time interval against a blank solution consisting of phosphate buffer as negative control. The percentage of scavenging of \( \text{H}_2\text{O}_2 \) against antioxidant was calculated as follows

The % of scavenged \( \text{H}_2\text{O}_2 \) = [(A0 - Ai)/ A0] X 100
Where A0 is the absorbance of control, Ai is the absorbance in the presence of the antioxidants.

The capacity of the antioxidants to scavenge \( \text{H}_2\text{O}_2 \) was determined at different concentration of antioxidants against different concentration of \( \text{H}_2\text{O}_2 \). The antioxidant property of the antioxidants was optimized against 3.5 mM \( \text{H}_2\text{O}_2 \) at 20 and 50 mg/ mL concentration.

**Statistical analysis**

The experiments were performed in triplicate. Data are expressed as mean ± standard deviation (SD).

**Results and Discussion**

**DPH radical scavenging activity**

The scavenging activity on DPH radicals is generally used as a basic screening method for testing the antiradical activity of a large variety of compounds [32]. DPH is a stable free radical that possesses a characteristic absorption maximum between 515 and 517 nm, which is diminished in the presence of a compound capable of reducing it to its hydrazine form by a hydrogen/electron transfer reaction [33]. To evaluate the antioxidant activity of the methanolic quince fraction extract, the radical scavenging capacity based on DPH assay was determined and the results are shown in (Figure 1) for the fractions. The scavenging activity showed a variable trend in different fractions of quince with peel showing highest (69.40%), followed by pulp (68.10%), seeds (55.90%) and lowest in mucilage (43.20%). The values obtained clearly reflect that the peel showed greater scavenging capacity, this might be due to presence of more phenolic compounds [9] and higher concentration of sugar acids (ascorbic acid). Mucilage was found to weak scavenging ability and it increased to lesser extent with increasing concentration of the extracts.

![Figure 1. DPPH value of different fractions of quince](attachment:image)

**Total phenolics**

The values obtained for total phenolic content in different fractions of quince are presented in (Figure 2). In case of different fractions it is evident from the figure 2 that peel possessed higher concentration (97.51 mg GAE/100 g) followed by seed (79.48 mg GAE/100g), pulp (41.23 mg GAE/100 g). The total phenolic values in the peels were higher than in the fresh pulps. In addition, [2] showed that quince peel extract had an approximately five-folder higher content of phenolics than that of the pulp. In earlier study, peel methanolic extract showed an EC\(_{50}\) of 600 µg/mL, followed by pulp and seed extracts, with EC\(_{50}\) of
1700 and 2000 µg/mL, respectively. Phenolic compounds might tend to accumulate in the dermal tissues of the plant body due to their potential role in protecting against ultraviolet radiations, acting as attractants in fruit dispersal, and as defence chemicals against pathogens and predators [34].

Figure 2. Total phenolic content of different fractions of quince

Reducing power

In the reducing power assay, all quince fraction extracts displayed a concentration-dependent antioxidant potential (Figure 3). In this assay, the presence of reducing agents in the extracts causes the conversion of the Fe3+/ferricyanide complex to the ferrous (Fe2+) form. Fe2+ is monitored by measuring the formation of Perl’s Prussian blue at 700 nm. The reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity [35]. The methanolic extracts of quince fraction displayed concentration dependent antioxidant potential in the reducing power assay. The results for the reducing power showed the same trend as was observed for the DPPH radical scavenging activity and total phenolic content. Among fractions, the highest value for reducing power was observed in peel (74.40%) and lowest value was seen in mucilage (31.90%), while as for pulp and seeds it was (65.50%) and (59.30%) respectively. These results showed methanolic extracts may act as an electron donor and therefore react with free radicals, convert them to more stable products and terminate radical chain reaction. The change in absorbance is therefore, directly related to the combined or “total” reducing power of the electron donating antioxidants present in the reaction mixture [36].

Figure 3. Reducing power of different fractions of quince

Ferric reducing antioxidant power (FRAP)

The reaction in the FRAP assay measures reduction of ferric 2, 4, 6-tripyridyl-s-triazine (TPTZ) to a colored product. The FRAP values obtained for different fractions of quince are presented in (Figure 4). From the figure it is evident that peel is having highest FRAP value of (1.16 µM), followed by pulp (1.12 µM), seeds (0.98 µM) and mucilage (0.63 µM). This is likely to be due to various types and amounts of antioxidants, including various types of phenolic compounds present in various concentrations in different fractions of quince which are responsible for the measured antioxidant activity. In various comparative studies all fruit peels possessed higher FRAP value than their pulps, with seeds showing variation in value [37].

Figure 4. FRAP value of different fractions of quince

H2O2 scavenging activity

The H2O2 scavenging (Hs) activity of different fractions of quince is presented in (Figure 5). With increase in concentration of the extract the H2O2 scavenging activity increased, as it is concentration dependent. The percentage of the H2O2 scavenging activity was (34.2%), (28.5%), (24.6%) and (14.2%) for peel, pulp, seeds and mucilage respectively. Peel is supposed to rich source of flavones, anthocyanins and various other phenolic compounds. The presences of these compounds in the peel are responsible for its higher H2O2 scavenging activity.
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Figure 5. $H_2O_2$ scavenging activity of different fractions of quince

Conclusion

*C. oblonga* fruit constitutes a promising natural source of bioactive compounds. Quince seeds and peels, often wasted in the production of quince products, deserve to be better exploited due to their beneficial properties. The antioxidant activities described for these materials may be indicative of the interest in quince fruits as a natural source of health promoting compounds, suitable for application in nutritional/pharmaceutical fields, in the prevention and treatment of free radical-mediated human chronic pathologies, such as cardiovascular diseases and cancer.

Conflict of interest

The authors declare that there is no conflict of interest to reveal.

References


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