## COMPARATIVE EVALUATION OF THE PROXIMATE COMPOSITION AND ANTIOXIDANT POTENTIALS OF QUINCE (*CYDONIA OBLONGA* MILLER.)

# SYED HASSAN ALI SHAH<sup>1</sup>, MOHAMMAD NISAR<sup>1</sup>\*, MOHAMMAD IHSAN<sup>1</sup>, MUHAMMAD ZAHOOR<sup>2</sup>, NAUSHEEN NAZIR<sup>2</sup> AND AMAL ALOTAIBI<sup>3</sup>

<sup>1</sup>Department of Botany, University of Malakand, Dir Lower 18800, Pakistan <sup>2</sup>Department of Biochemistry, University of Malakand, Dir Lower 18800, Pakistan <sup>3</sup>Department of Basic Science, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, 11671, Saudi Arabia \*Correspondence: mohammadzahoorus@yahoo.com

#### Abstract

This study was aimed to determine the proximate composition and antioxidant potentials of Quince genotypes. The proximate composition was estimated using the reported literature methods whereas the antioxidant potentials were determined using DPPH and ABTS assays. The possible phyto-constituents were identified using an HPLC Agilent 1260 system. The highest protein contents were present in genotype collected from Nalkot whereas lowest in Dardyal genotype. In leaves protein ranged from 0.41 to 0.6%. Crude fibers in fruit ranged from 13.89 % to 19.5 %, with highest contents in Malam Jaba genotype whereas, in leaves they were estimated from 14.83% to 19.69%. Ash content of the fruit ranged from 3.09% to 5.4% and in leaves from 3.91% to 7.3%. The highest carbohydrate value was recorded for Nalkot genotype. Fats (lipids) in fruit ranged from 0.38% to 0.93% whereas in leaves they ranged from 0.27% to 0.81% with highest contents in Charbagh and Tindodag genotypes. The estimated moisture contents in fruit were 55.69% to 68.73% whereas in leaves moisture contents ranged from 55.44% to 73.88%. The HPLC analysis revealed a numbered of compound peaks however only five of them were identified following spiking technique (Malic acid, Galic acid, Mandelic Acid, Phloroglucinol and Hydroxy Benzoic acid). The DPPH radical inhibitions of fruit extracts were;  $79\pm0.1$ ,  $66\pm0.5$ ,  $64\pm1.2$ ,  $60\pm1.1$ ,  $62\pm1.5$ ,  $64\pm0.1$ , and  $66\pm1.4$  of the tested concentrations. The fruit sample collected from Tindodag genotype caused significant inhibition in comparison to standard ascorbic acid. The leaves samples inhibited the DPPH free radical by  $62\pm1.2$ ,  $55\pm1.4$ ,  $60\pm1.5$ ,  $65\pm1.8$ ,  $62\pm1.4$ ,  $640\pm1.2$ , and  $60\pm1.8$ . The extent of ABTS inhibition were;  $76\pm0.1$ ,  $68\pm1.5$ ,  $66\pm1.2$ ,  $55\pm1.3$ ,  $60\pm1.5$ ,  $66\pm0.5$ , and  $63\pm0.5$  for fruit samples whereas for leaves samples the recorded values were; 60± 0.5, 57±1.5, 63±.0.2, 63±.0.5, 60±1.2, 65± 0.5, and 55±0.2. Tindodag genotype fruit and leaves have caused significant inhibition as compared to the used standards. This study suggests that cultivation of Tindodag genotype in other areas might be encouraged to get highly nutritious fruit having health benefits as well.

Key words: Quince, Mineral composition, HPLC finger printing, Antioxidant.

#### Introduction

Fruits and vegetables are rich sources of vitamins and minerals. They are not only sources of nutrients and minerals but they also contain millions of phytochemicals falling under the category of secondary metabolites. The secondary metabolites are prepared by plants as tools of offense and defense therefore, even same plant from one area will have different phytochemical composition from the one growing in another locality. The secondary metabolites are categorized as alkaloids, tannin, terpenoids, phenolics, flavonoids etc. The phenolics and flavonoids have been reported to have strong antioxidant potentials. Fruits are usually rich in dietary fibers and relatively low in calories (Fulton et al., 2016). By virtue of their secondary metabolite composition, fruits and vegetables are considered to be beneficial in reducing the risks of some chronic diseases (Li et al., 2017) like cardiac diseases and even cancer (Entesar et al., 2020).

Quince (*Cydonia oblonga* M.) is a small tree or shrub and is native to Turkey and Iran (Sharma *et al.*, 2011). It has simple pome fruit and belongs to Rosaceae family (Entesar *et al.*, 2020). Quince is planted in warm temperate regions and it grows to 4 meter in width and 8 meter in height. Pale greyish wool covers the young branchlets of Quince. They have elliptical leaves and pink or white flowers (Maryam & Abolghasem, 2011). In fruiting season, Quince bears fragrant apple or pear-shaped fruits, which are juicy and golden yellowish in color (Entesar *et al.*, 2020). The fruit contains large number of seeds which are coated with mucilage (Entesar *et al.*, 2020). Peel of the fruit is covered with hairs which falls when the fruit ripens (Entesar *et al.*, 2020). Quince fruit is a good source of flavonoids and phenolic compounds (Mir *et al.*, 2015). It also contained a substantial amount of poly-saccharides in its cell wall, (Entesar *et al.*, 2020).

Experimentally it has been found that primary metabolites, elemental and phytochemical composition among different genotypes of the same species growing in different localities, are different (Ibrahim et al., 2020). Such differences in phytochemical and nutritional compositions arise due to the differences in their genetic makeup called genetic diversity. There are several ways to determine genetic diversity among different cultivars and genotypes. Comparison of proximate compositions is one of the best options to select germplasms as future cultivar having high nutritional values (Ibrahim et al., 2020). Quince fruit has high nutritional values and has positive effect on human health (Sharma et al., 2011). It has been found that Quince fruit is composed of 90.6% of pulp, 4.4% peel and 5% seed with core. Quince juice contains 1.2% malic acid and also small amount of tartaric acid. Its fruit is also good source of ascorbic acid and minerals such as phosphorous, potassium and calcium (Sharma et al., 2011). Quince fruit is rich in protein, pectin, organic acids, amino acids, phenolics, volatile/essential oils, flavonoids,

vitamins, natural wax and terpenoids (Hopur *et al.*, 2012). It also contains valuable monosaccharide as part of their polysaccharides that are associated with health benefits and are pharmaceutically important (Hopur *et al.*, 2012).

Quince plant has been ignored for last two decades and there is a potential risk of its extinction in next few decades. To help the plant survival, documentation of its health benefits and nutritional values is very important. Keeping in view the mentioned risks, the present study was designed to evaluate the proximate composition of Quince genotypes to suggest a better variety for future cultivation by farmers and revive its best health benefits. The phytochemical composition of this plant was evaluated using HPLC analysis whereas its antioxidant potentials were estimated using DPPH and ABTS assays.

#### **Material and Methods**

**Plant sample:** Fruit and leaves samples of Quince plant were collected from different areas of Khyber Pakhtunkhwa including Charbagh, Nalkot, Malamjabba, Tindodag, Chitral, Dardyal and Talash. Fruit and leaves were brought to University of Malakand, cleaned and dried which were then subjected to proximate analysis, HPLC analysis and antioxidant evaluation following standard protocols.

Proximate analysis: For nutritional analysis, fruit and leaves samples of Quince were analyzed for contents like moisture, crude lipids, crude fibers, crude protein, ash and carbohydrates following the standard procedures of ACOC (Horwitz, 2010). About 100 g of fruit and leaves samples were grounded into fine powder and then subjected to proximate analysis. In order to determine moisture contents, 3 g of the sample was taken in a pre-weighed petri-dish. Then the sample was dried for 6 h at 100°C in an oven. The dish containing the sample was cooled in desiccators and difference between pre and post weights were noted as moisture contents. To measure ash contents, 1 g of powdered sample was taken in a crucible and placed in a muffle furnace at 550°C for 5 h. The crucible was weighed after cooling and ash contents were estimated by subtracting the crucible weight from it. To measure protein content, 1 g of sample was digested with conc. H<sub>2</sub>SO<sub>4</sub> in the presence of Na<sub>2</sub>SO<sub>4</sub> and CuSO<sub>4</sub> and then heated. The ammonia produced was then steam distilled into a boric acid solution. The ammonia nitrogen was calculated by titration of the trapped ammonia with 0.1 M HCl (double indicator) in the presence of Tashirus indicator until the observance of a purple-pink color. The estimated nitrogen value was then multiplied by 6.25 mg to get crude protein values. To calculate fiber contents, 50 ml of 1.25% H<sub>2</sub>SO<sub>4</sub> was used to digest 2 g of powdered sample by boiling for 30 min. The mixture was further digested with 50 ml of 1.25% NaOH for 30 min, then the mixtures were dried and heated at 550°C in a furnace. The residue left after ignition were then weighed and used for the estimation of fiber contents. For the estimation of fat contents, 1 g of sample was extracted with 10 ml of ether and placed in an

incubator at 40°C for 12 h in test tubes till complete evaporation. The upper layer of the solution was then transferred into graduated tubes which were then again dried in oven at 40°C for 4 h. The weight difference between pre and post evaporation in the tube contents were measured as fat contents. Carbohydrates contents were then calculated using following relation:

Carbohydrate contents = 100 - (Protein % + Ash % + Moisture % + Fat %) (1)

**HPLC-UV sample preparation:** HPLC analysis were performed for 7 samples using an Agilent 1260 system. The powdered samples of Quince fruit were digested with methanol and water (1: 1, 20ml, v/v). For 1 h, the mixture was heated at 70°C in the water bath and then it was centrifuged at 4000 rpm for 10 min. After that, 2 ml of sample was filtered through Whatman filter paper into HPLC vials. The vials were then labeled with proper codes. The phenolic compounds were then identified through comparison of sample's retention time and that of available standards.

DPPH free radical scavenging assay: Brand William assay (Brand-William et al., 1995) was followed to determine the antioxidant activity of Quince methanolic extracts of fruit and leaves. The DPPH (2, 2-Diphenyl-1picrylhydrazyl) solution in 100 ml methanol was prepared by dissolving 2 mg DPPH in methanol. The stock solutions of Quince fruit and leaves were prepared in methanol, having a concentration of 1 mg/ml. These stock solutions were further diluted to the concentrations of 1000, 500, 250, 125 and 62.5 µg/ml. About 1 ml diluted solution of each sample was then mixed with 3 ml of DPPH solution. It was then incubated for 30 min at 25°C. Finally, the absorbance was measured at 517 nm with the help of UV spectrophotometer. Ascorbic Acid was used as positive control. Each test was performed in triplicate. The percent inhibition of each sample was calculated using relation:

% Inhibition = 
$$\frac{A-B}{A} \times 100$$
 (2)

where A is the absorbance of pure DPPH in oxidized form and B is the absorbance of the sample after DPPH addition, which was measured after 15 min of incubation.

ABTS (2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) free radical assay: The reported assay of Re *et al.*, (1999) was used to calculate ABTS free radicals' inhibition potential of extracts. Solution of ABTS was prepared as per the reported protocol of Re *et al.*, (1999). These stock solutions were further diluted to the concentrations of 1000, 500, 250, 125 and 62.5  $\mu$ g/ml. Then 300  $\mu$ L samples of Quince fruit and leaves' methanolic extracts were thoroughly mixed with 3 ml ABTS solution. The mixtures were incubated for 6 min and the absorbance of mixtures was measured through UV spectrophotometer. Ascorbic acid was used as positive control. Relation 2 was used to estimate percent inhibitions.

## Results

**Proximate analysis:** Quince fruit and leaves collected from seven different places, were analyzed for their proximate composition. The experiments were conducted in three replicas. Mean and standard deviation tools were used to statistically analyze the data. The data of proximate composition of fresh fruit and leaves are presented in Table 1. The fruit protein contents ranged from 0.42% to 0.76%. Highest values of 0.76% was recorded for Nalkot genotype whereas lowest contents were recorded for Dardyal genotype (0.42%). In leaves, protein contents ranged from 0.41% to 0.6%; highest in Talash genotype (0.6%). Crude fibers in fruit were estimated in range of 13.89% to 21.84% with highest value in Talash genotype. In leaves, the examined crude fibers ranged from 14.83% to 19.69% and here also Talash genotype was ranked high. Ash content of

fruit was measured to be in range of 3.09% to 5.4%, whereas in leaves it was measured as 3.91% to 7.3% with highest contents in Charbagh genotype (5.4% and 7.3% in fruit and leaves respectively). Carbohydrates were ranged from 8.95% to 21.74% in fruit samples whereas leaves contained carbohydrates from 3.28% to 24.18%. Carbohydrates with highest values of 21.74% and 24.18% respectively, were recorded in fruit and leaves of Nalkot genotype. Fats contents in fruit ranged from 0.38% to 0.93% and in leaves its range was from 0.27% to 0.81%. Highest fats contents were examined in Charbagh genotype's fruit sample and leaves sample of Tindodag genotype. The examined moisture contents in fruit ranged from 55.69% to 68.73% whereas in leaves its range was from 55.44% to 73.88%. Highest moisture was recorded in fruit of Tindodag genotype (68.73%) and in leaves of Charbagh genotype (73.88%). Results are tabulated in table 1.

|               | • • • • • • • • • • • • • • • • • • • | Mean + Standard deviation            | Mean + Standard deviation             |  |
|---------------|---------------------------------------|--------------------------------------|---------------------------------------|--|
| Parameters    | Genotypes                             | (Fruit)                              | (Leaves) (%)                          |  |
| Protein       | Charbagh                              | $0.71 \pm 0.17$                      | $0.55 \pm 0.04$                       |  |
|               | Dardyal                               | $0.42 \pm 0.05$                      | $0.42 \pm 0.04$                       |  |
|               | Tindodag                              | $0.67 \pm 0.06$                      | $0.43 \pm 0.05$                       |  |
|               | Chitral                               | $0.63 \pm 0.05$                      | $0.49 \pm 0.06$                       |  |
|               | Malam Jabba                           | $0.46 \pm 0.06$                      | $0.58 \pm 0.05$                       |  |
|               | Talash                                | $0.58 \pm 0.07$                      | $0.60 \pm 0.07$                       |  |
|               | Nalkot                                | $0.76 \pm 0.05$                      | $0.41 \pm 0.06$                       |  |
| Crude fiber   | Charbagh                              | $13.89 \pm 1.51$                     | $16.54 \pm 0.94$                      |  |
|               | Dardyal                               | $17.87 \pm 0.45$                     | $19.09 \pm 2.49$                      |  |
|               | Tindodag                              | $17.67 \pm 0.43$<br>$18.29 \pm 1.63$ | $19.09 \pm 2.49$<br>$18.72 \pm 0.35$  |  |
|               | Chitral                               | $20.66 \pm 1.64$                     | $16.72 \pm 0.035$<br>$16.27 \pm 0.46$ |  |
|               | Malam Jabba                           | $19.5 \pm 0.15$                      | $10.27 \pm 0.40$<br>$19.69 \pm 1.00$  |  |
|               | Talash                                | $21.84 \pm 2.99$                     | $17.60 \pm 0.67$                      |  |
|               | Nalkot                                | $15.73 \pm 1.65$                     | $17.00 \pm 0.07$<br>$14.83 \pm 1.43$  |  |
|               | Charbagh                              | $5.40 \pm 0.10$                      | $7.30 \pm 0.72$                       |  |
|               | Dardyal                               | $3.40 \pm 0.10$<br>$3.93 \pm 0.44$   | $7.30 \pm 0.72$<br>$6.64 \pm 0.65$    |  |
|               | Tindodag                              | $3.95 \pm 0.44$<br>$3.09 \pm 0.17$   |                                       |  |
| Ash           | Chitral                               |                                      | $4.36 \pm 0.66$                       |  |
| ASII          | Malam Jabba                           | $4.26 \pm 0.36$                      | $3.91 \pm 0.59$                       |  |
|               |                                       | $3.54 \pm 0.36$                      | $5.82 \pm 0.40$                       |  |
|               | Talash                                | $4.35 \pm 0.81$                      | $5.50 \pm 0.31$                       |  |
|               | Nalkot                                | $3.62 \pm 0.55$                      | $4.73 \pm 0.60$                       |  |
|               | Charbagh                              | $14.35 \pm 1.47$                     | $11.30 \pm 1.5$                       |  |
|               | Dardyal                               | $15.37 \pm 1.99$                     | $7.94 \pm 0.99$                       |  |
| a 1 1 1       | Tindodag                              | $8.95 \pm 1.08$                      | $10.35 \pm 0.67$                      |  |
| Carbohydrates | Chitral                               | $15.18 \pm 0.99$                     | $6.02 \pm 0.87$                       |  |
|               | Malam Jabba                           | $15.59 \pm 0.62$                     | $3.28 \pm 0.72$                       |  |
|               | Talash                                | $15.71 \pm 1.05$                     | $17.67 \pm 0.87$                      |  |
|               | Nalkot                                | $21.74 \pm 1.01$                     | $24.18 \pm 1.76$                      |  |
|               | Charbagh                              | $0.93 \pm 0.09$                      | $0.64 \pm 0.11$                       |  |
|               | Dardyal                               | $0.75 \pm 0.21$                      | $0.57 \pm 0.05$                       |  |
|               | Tindodag                              | $0.48\pm0.08$                        | $0.81 \pm 0.05$                       |  |
| Fats          | Chitral                               | $0.65 \pm 0.08$                      | $0.74 \pm 0.04$                       |  |
|               | Malam Jabba                           | $0.73 \pm 0.12$                      | $0.47 \pm 0.05$                       |  |
|               | Talash                                | $0.54 \pm 0.10$                      | $0.39 \pm 0.03$                       |  |
|               | Nalkot                                | $0.38 \pm 0.03$                      | $0.27 \pm 0.08$                       |  |
| Moisture      | Charbagh                              | $64.12 \pm 3.35$                     | $72.74 \pm 8.35$                      |  |
|               | Dardyal                               | 61.95 <u>+</u> 3.65                  | $67.74 \pm 2.15$                      |  |
|               | Tindodag                              | $68.37 \pm 2.83$                     | $65.82 \pm 2.70$                      |  |
|               | Chitral                               | $58.66 \pm 3.72$                     | $73.88 \pm 3.11$                      |  |
|               | Malam Jabba                           | $60.82 \pm 2.57$                     | $56.93 \pm 20.77$                     |  |
|               | Talash                                | $55.69 \pm 3.50$                     | $58.52 \pm 1.15$                      |  |
|               | Nalkot                                | $57.56 \pm 4.28$                     | $55.44 \pm 2.01$                      |  |

Table 1. Proximate compositions of fruit and leaves samples of Quince genotypes.

|            | Retention time | <b>A V</b>           |      | HPLC-UV Amax Peak area of Identification |           |  |
|------------|----------------|----------------------|------|--|-----------|--|
| Genotypes  | (min)          | Phenolic compounds   | (nm) | sample                                   | reference |  |
|            | 2.263          | Malic acid           | 320  | 936.042                                  | Standard  |  |
| Talash     | 3.516          | Galic acid           | 320  | 243.224                                  | Standard  |  |
|            | 32.890         | Phloroglucinol       | 320  | 45.293                                   | Standard  |  |
|            | 35.441         | Hydroxy Benzoic acid | 320  | 25.961                                   | Standard  |  |
| Nalkot     | 2.295          | Malic acid           | 320  | 1258.731                                 | Standard  |  |
|            | 3.515          | Galic acid           | 320  | 318.178                                  | Standard  |  |
|            | 32.887         | Mandelic Acid        | 320  | 44.459                                   | Standard  |  |
|            | 35.913         | Hydroxy Benzoic acid | 320  | 124.727                                  | Standard  |  |
| Tindodag   | 2.288          | Malic acid           | 320  | 168.82445                                | Standard  |  |
|            | 3.525          | Galic acid           | 320  | 211.26521                                | Standard  |  |
|            | 32.916         | Mandelic Acid        | 320  | 81.52232                                 | Standard  |  |
|            | 34.319         | Phloroglucinol       | 320  | 109.00055                                | Standard  |  |
|            | 35.032         | Hydroxy Benzoic acid | 320  | 149.76308                                | Standard  |  |
| Charbagh   | 2.310          | Malic acid           | 320  | 99.06074                                 | Standard  |  |
|            | 3.523          | Galic acid           | 320  | 229.15302                                | Standard  |  |
|            | 32.905         | Mandelic Acid        | 320  | 106.12796                                | Standard  |  |
|            | 34.297         | Phloroglucinol       | 320  | 149.27                                   | Standard  |  |
|            | 35.475         | Hydroxy Benzoic acid | 320  | 26.17837                                 | Standard  |  |
| Chitral    | 2.322          | Malic acid           | 320  | 198.80870                                | Standard  |  |
|            | 3.525          | Galic acid           | 320  | 430.37231                                | Standard  |  |
|            | 34.263         | Phloroglucinol       | 320  | 25.47190                                 | Standard  |  |
|            | 35.903         | Hydroxy Benzoic acid | 320  | 123.90078                                | Standard  |  |
| Malam Jaba | 2.258          | Malic acid           | 320  | 1667.80273                               | Standard  |  |
|            | 3.303          | Galic acid           | 320  | 1723.07324                               | Standard  |  |
|            | 34.304         | Phloroglucinol       | 320  | 105.73909                                | Standard  |  |
|            | 35.980         | Hydroxy Benzoic acid | 320  | 125.56604                                | Standard  |  |
| Dardyal    | 2.495          | Malic acid           | 320  | 42.88372                                 | Standard  |  |
|            | 3.238          | Galic acid           | 320  | 165.56799                                | Standard  |  |
|            | 34.332         | Phloroglucinol       | 320  | 107.53027                                | Standard  |  |
|            | 35.531         | Hydroxy Benzoic acid | 320  | 26.09601                                 | Standard  |  |

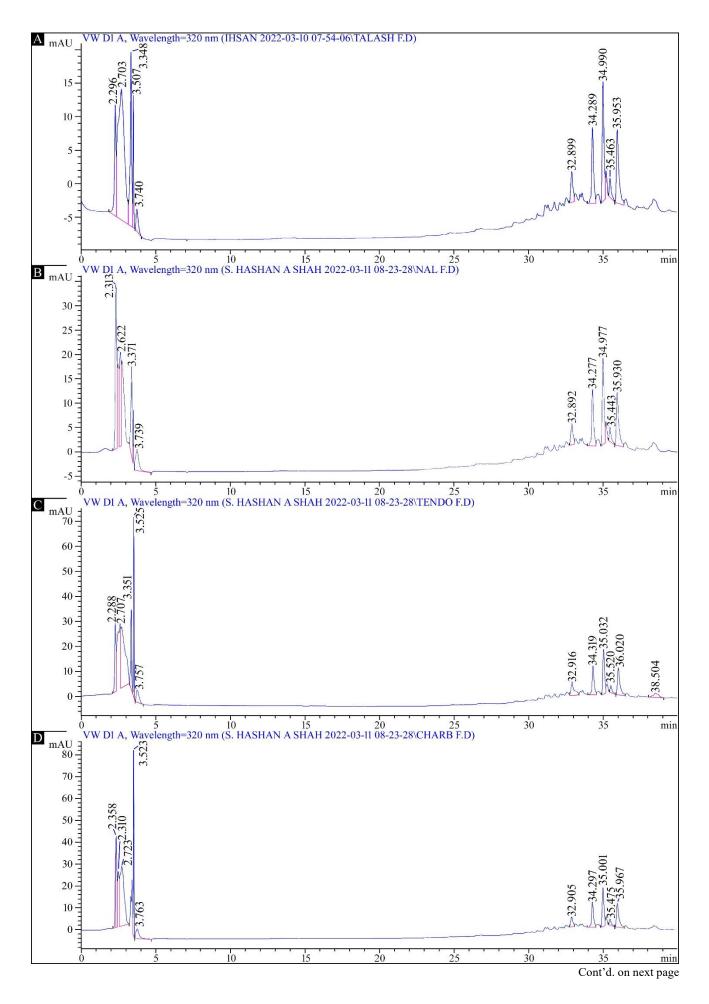
Table 2. Identified phytochemicals in Quince genotypes fruit samples

Identification of phenolic compounds in Quince genotypes using HPLC-UV analysis: Figure 1, presents HPLC-UV chromatograms of fruit samples whereas Table 2 shows the identified compounds and their quantification with their specific peak position and retention time (Rt) in chromatogram. All these phenolic compounds were identified by comparing their retention times of the phenolic compounds and confirmed them through spiking with standards. In Talash genotype, 4 phytochemicals, namely Malic acid, Galic acid, Phloroglucinol and Hydroxy Benzoic acid were identified (Fig. 1A). Four compounds Malic acid, Galic acid, Mandelic Acid and Hydroxy Benzoic acid were observed in the extract of Nalkot genotype (Fig. 1B). Five compounds Malic acid, Galic acid, Mandelic Acid, Phloroglucinol and Hydroxy Benzoic acid were identified in Tindodag genotype (Fig. 1C). Similarly, in Charbagh genotype a total of five compounds namely Malic acid, Galic acid, Mandelic Acid, Phloroglucinol and Hydroxy Benzoic acid were identified as present in Fig. 1D. Four compounds (Malic acid, Galic acid, Phloroglucinol and Hydroxy Benzoic acid) were identified in Chitral, Malamjaba and Dardyal genotypes as presented in Figs. 1E-1G.

*In vitro* **DPPH free radicals scavenging activities:** Figure 2, shows the antioxidant potential of fruit and leaves

samples of Quince plants collected from various localities. Tindodag, Talash, Nalkot, Dardyal, Malamjaba, Charbagh, and Chitral. Fruit samples inhibited the DPPH free radical with percent inhibition of 79 $\pm$ 0.1, 66 $\pm$ 0.5, 64 $\pm$ 1.2, 60 $\pm$ 1.1, 62 $\pm$ 1.5, 64 $\pm$ 0.1, and 66 $\pm$ 1.4 respectively, at the highest concentration tested (1000 µg/mL) with Tindodag sample ranked to be highly effective in comparison to standard ascorbic acid (Fig. 2A). The leaves samples of Tindodog, Talash, Nalkot, Dardyal, Malamjaba, Charbagh, and Chitral in form of extracts inhibited the DPPH free radicals by percent inhibition of 62 $\pm$ 1.2, 55 $\pm$ 1.4, 60 $\pm$ 1.5, 65 $\pm$ 1.8, 62 $\pm$ 1.4, 64 $\pm$ 1.2, and 60 $\pm$ 1.8 respectively, at the highest tested concentration of 1000 µg/mL (Fig. 2B).

*In vitro* **ABTS free radicals scavenging activity:** Figure 3, shows the antioxidant potential of fruit and leaves extracts of Quince. Tindodag, Talash, Nalkot, Dardyal, Malamjaba, Charbagh, and Chitral Fruit extracts have inhibited the ABTS free radicals by percent inhibition of 76±0.1, 68±1.5, 66±1.2, 55±1.3, 60±.1.5, 66±0.5, and 63±0.5 respectively, at the highest tested concentration of 1000 µg/mL (Fig. 3A) while the corresponding leaves extracts inhibited the ABTS free radicals by percent inhibition of 60± 0.5, 57±1.5, 63±.0.2, 63±.0.5, 60±1.2, 65± 0.5, and 55±0.2 respectively, at the mentioned highest concentration.



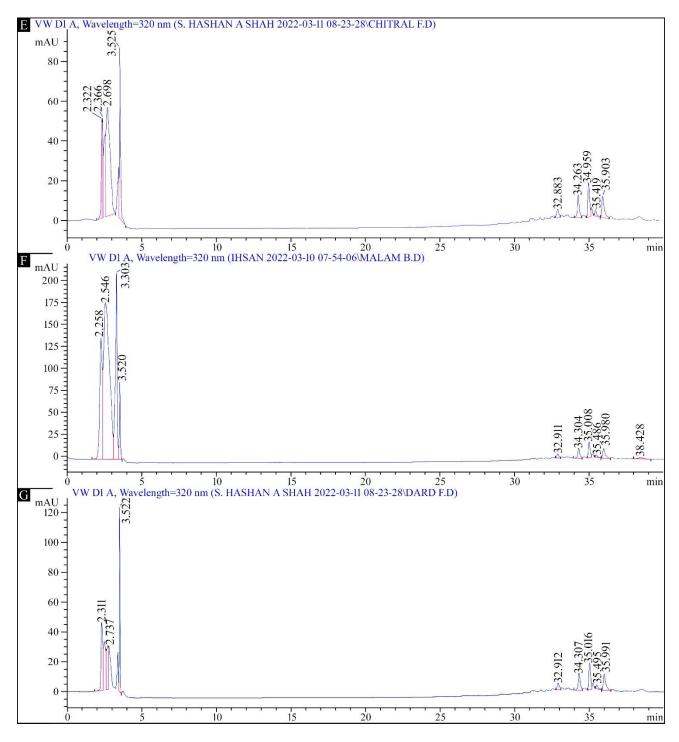


Fig. 1. HPLC-UV chromatograms of Quince fruit extracts; Talash (A), Nalkot (B), Tindodag (C) and Charbagh (D), Chitral (E), Malamjaba (F) and Dardyal (G).

#### Discussion

Quince (*Cydonia oblonga*) fruit is popular among local community however, not grown on large commercial scale. To help its propagation on large scale, the present study was conducted. This fruit has positive influence on human health because of its high nutritional value and associated health benefits. Its various nutritional and phytochemical properties, from dietary utilization to medicinal importance, have made this plant a best nutritional and medicinal candidate to be explored in detail. The phytochemicals present in this fruit not only plays important role in maintaining of fruit quality, nature, behavior, and their response to handling and processing but are also source of medication. Fruit and leaves of 7 different genotypes of quince were analyzed for different phytochemical and nutritional (proximate) composition. In addition, these genotypes were also analyzed for their antioxidant potentials estimated through DPPH and ABTS assays.

Nutritional analysis helps us to select a proper genotype for future growth which is best in nutritional value if used as diet. Protein, fibers, lipids, carbohydrates, ash and moisture contents were analyzed for the collected fruit and leaves samples. Protein is included as a major component in any healthy diet which provides amino acids for the wear and tear processes going on in the human bodies. Although, almost all fruits are low in protein contents yet they can add a share of few calories. Protein content in the leaves of Charbagh. Chitral. Tindodag and Nalkot genotypes were high as compared to their respective fruit samples. In Dardyal genotype, protein contents were equal in leaves and fruit samples. Malam Jabba and Talash genotypes' fruit samples were unexpectedly high in protein contents as compared to their leaves samples. Leaves of Nalkot has high content of protein (0.76%), while leaves of Malam Jabba has least protein content (0.46%) among all the studied genotypes. Talash genotype has highest content of protein in their fruit (0.60%) while Nalkot genotype has lowest value of protein content (0.41%). Similar results were previously reported by Rasheed et al., (2018) and Sood & Bhardwaj (2015). Hegedus et al., (2013) have reported that Quince has more proteins than apple. About 0.39% protein contents were recorded in another reported study of Faizullah et al., (2023).

The results in a reported study of Silva et al., (2007) have shown that Quince is rich in fibers associated with wide range of health benefits. It lowers cholesterol level and also maintains blood sugar level. They are also helpful in digestion. Crude fibers in leaves of all genotypes of Quince, except Chitral and Dardyal genotypes, were higher than fruit. Crude fiber in leaves of Talash genotype (21.84%) was recorded as highest among all genotypes while the leaves of Charbagh genotype contained its lowest content (13.89%). Crude fiber in fruit of Malam Jabba genotype were high (19.69%) as compared to other genotypes, while lowest in Nalkot genotype (14.83%). Sood & Bhardwaj (2015) recorded 5.7% crude fibers in Quince whereas Faizullah et al., (2023) reported 4.9% crude fibers. This shows that genotypes of Quince found in Pakistan are rich in fiber content than the mentioned two reported studies. In another study of Hegedus et al., (2013) it has shown that Quince is rich in fiber content than apple which they estimated as 1.9 g of fiber in Quince while 1.3 g in apple, suggesting that Quince is good source of fiber as compared to apple.

Carbohydrates are the most important and readily available source of energy for human body. They are one of the three important nutrients found in fruit and vegetables. Fruit has natural sugar which adds to daily carbohydrate count of the body. Fruit of Talash and Nalkot genotypes have highest carbohydrates than their corresponding leaves, while all other genotypes have less carbohydrates in their fruit in comparison to their leaves. Carbohydrates were recorded highest in fruit of Nalkot genotype (24.18%) while lowest in Malam Jabba genotype (3.28%). Leaves of Nalkot genotype exhibited highest carbohydrate contents (21.74%) while leaves of Tindodag genotype have lowest carbohydrates (8.95%). In a reported study 9.3% carbohydrates in Quince have been shown (Faizullah et al., 2023), showing that Pakistani varieties are rich in carbohydrates. Hegedus et al., (2013) have reported that Quince is rich in carbohydrates in comparison to apple and according to them 9.1 g carbohydrates were recorded in Quince and 7 g in apple.

Lipids, that are obtained from plants, are good source of essential fatty acids. It also has a crucial role in flavor development. Lipids or fat content was found higher in leaves than in fruit samples of the studied genotypes, except Chitral and Tindodag genotypes where lipid contents were high in fruits than leaves. Highest lipids content was recorded in Tindodag genotype's fruit (0.81%) and lowest in Nalkot genotype's fruit (0.27%). Leaves of Charbagh genotype possessed highest content of lipids (0.93%) and Nalkot genotype was ranked as lowest in lipids (0.38%). Nalkot genotype exhibited lowest content of lipids in their leaves and fruit. Rasheed *et al.*, (2018) recorded 0.24% of lipids in Quince, which are in accordance with our findings. About 0.06% lipid contents have been reported by Faizullah *et al.*, (2023).

Moisture content is very important factor while describing fruit quality as it maintains its freshness for long time. Highest level of moisture was recorded for Chitral genotype as 73% and 58% in fruit and leaves respectively. Lowest level of moisture was recorded in fruit sample of Nalkot genotype (55.44%) and leaves of Talash genotype (55.69%). Sood & Bhardwaj (2015) have reported 82% moisture content whereas Rasheed *et al.*, (2018) reported it to be 84.27% in Quince fruit. Hegedus *et al.*, (2013) have reported 86.9% of moisture which was lower than apple (90.5%). About 84.1% moisture content was recorded in a study conducted by Faizullah *et al.*, (2023).

Ash is the inorganic residue left behind when organic matter is burnt. It shows us total amount of minerals present in a given food. Ash contents were minimum in fruit samples than in leaves of all genotypes. Charbagh genotype has highest content of ash both in their fruit and leaves which contain 7.3% and 5.4% ash respectively. Lowest content of ash was recorded in fruit (4.36%) and leaves (3.09%) samples of Tindodag genotype. Rasheed *et al.*, (2018) recorded 0.62% ash content whereas Sood & Bhardwaj (2015) recorded 2.5%, while 3.18% of ash content was recorded by Faizullah *et al.*, (2023) in Quince fruit. Hegedus *et al.*, (2013) have reported that Quince has more ash content (0.6 g) than apple (0.4g).

HPLC analysis revealed the presence of 5 different phytochemicals in the fruit sample. In Charbagh and Tindodag genotypes; Malic acid, Galic acid, Mandelic Acid, Phloroglucinol and Hydroxy Benzoic acid were present, while in the remaining genotypes 4 compounds were identified as listed in Table 2. The HPLC results of Hopur et al., (2012) explians that Quince polysaccharides are mainly composed of Ara and Gal. Silva et al., (2007) reported many phenolic compounds like 3-Ocaffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid and many other unidentified phenolic compounds in their study. The present investigation is comparable with that of Ihsan et al., (2022), where they studied different Oat genotypes and recorded a total of 9 phytochemical compounds. The identified compounds have different health advantages if used by human for treating different diseases (Nazir et al., 2020).

The antioxidant potentials of the fruit and leaves samples of Quince were evaluated by DPPH and ABTS methods. Methanolic extracts of the collected samples were used as inhibitors of the tested free radicals. The results showed highest antioxidant potential for all the collected samples. It shows that Quince fruit would be beneficial in relieving oxidative stress. The results of Muzykiewicz *et al.*, (2018) and Costa *et al.*, (2009) have shown that Quince fruit has highest antioxidant potential which are in close agreement with our study. They have pointed out that leaves have high antioxidant values than fruit. In contrast, the current study shows high antioxidant potentials of fruit than leaves which might be sourced from the fact that fruit have been harvested in unripe condition in the sampled areas of this research. It is a fact that unripe fruits are rich in phytochemicals as compared to fully ripen fruits. The results of (Nazir *et al.*, 2020) justify that unripe fruit have more antioxidant potential than ripen fruit.

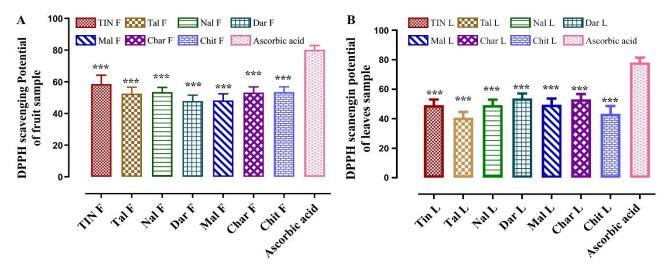


Fig. 2. Percent *In vitro* DPPH free radicals scavenging activity of fruit (A) and leaves (B) sample of Quince plant collected from different localities. **{(A)** % DPPH scavenging activity of fruit sample **(B)** % DPPH scavenging activity of leaves sample of Quince plant at various concentrations. Data are expressed as mean  $\pm$  SEM, \*\*p<0.01, \*\*\*p<0.001; comparison of Plant extract samples vs positive control Ascorbic acid}.

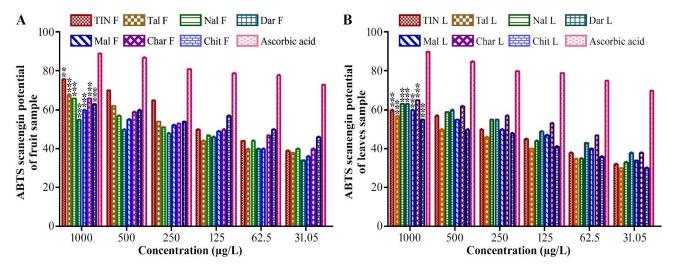


Fig. 3. Percent *In vitro* ABTS scavenging activity of Fruit (A) and leaves (B) sample of Quince plant collected from different localities.  $\{(A) \ \% \ ABTS \ scavenging \ activity \ of \ fruit \ sample \ (B) \ \% \ ABTS \ scavenging \ activity \ of \ leaves \ sample \ of \ Quince \ plant \ at \ various \ concentrations. Data are expressed as mean <math>\pm \ SEM, \ **p<0.01, \ ***p<0.001; \ comparison \ of \ test \ samples \ (Plant \ extract \ samples) \ vs \ positive \ control \ Ascorbic \ acid \.$ 

#### Conclusions

Quince (*Cydonia oblonga*) is rich in bioactive compounds and nutrients. It is evident from the results of this study. Quince is a good supplement to be added in daily nutrition as it contains all essential components like protein, carbohydrates, lipids, and other secondary metabolites. The fiber contents are also high in Quince, which points towards its health benefits. Quince fruit, in raw form, is not preferably eaten because of its astringent taste, that is why it is processed into number of value-added products like jams and jellies. This study highlights a significant amount of secondary metabolites in Quince, particularly Malic acid, Hydroxy-benzoic acid and Gallic acid which have put immense share in the recorded antioxidant potential. It is suggested that Quince plant should be further investigated for its medicinal values such as anti-bacterial and anti-fungal activities.

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