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PROXIMATE, MINERAL ANALYSIS AND ANTIOXIDANT ACTIVITIES OF SELECTED WILD FRUITS FROM DISTRICT KURRAM KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

Wild edible fruits play an important role in the nutritional security of humans around the globe. The detailed analyses of nutritional and minerals composition of wild fruits of the Kurram region (in Pakistan) are lacking. The objective of the current study was to determine the nutritional, minerals composition and antioxidants activities of five selected wild fruits *Rubus fruticosus, Prunus jacquemontii, Nannorrhops ritchiana, Crataegus oxyacantha,* and *Elaeagnus angustifolia*. Among these wild fruits the highest moisture content (27.1%) was recorded in *Nannorrhops ritchieana* while crude protein was at a maximum in *Nannorrhops ritchiana* (11.4%) and in *Prunus jacquemontii* (11.3%), while ash was highest (15.4%) in *Prunus jacquemontii*. On the other hand, crude fat (11.7%) was the maximum in *Prunus jacquemontii*, crude fiber was found to be the higher (13.4%) in *Rubus fruticosus*, and the maximum carbohydrate (85%) was found in *Elaeagnus angustifolia*. Notable amounts of macro minerals like potassium was found to be a maximum in *Crataegus oxyacantha* (77.2mg/L), calcium in *Elaeagnus angustifolia* (102.7mg/L), and magnesium in *Rubus fruticosus* (13.7 mg/L) as well as in *Nannorrhops ritchiana* (13.6 mg/L). The microelements like iron were recorded to be a maximum (3.4 mg/L) in *Prunus jacquemontii*, zinc as a maximum (0.44 mg/L) in *Nannorrhops ritchiana*, both cobalt and manganese concentrations of (0.3 mg/L), (0.2mg/L) were observed to be the highest in *Rubus fruticosus* respectively, while chromium were found to be a maximum in *Elaeagnus angustifolia* i.e. 0.07mg/L. Sodium was found to be high in *Nannorrhops ritchiana* (6.01mg/L). Highest FRSA% was reported in *Rubus fruticosus* which was 77% and TAC% was maximum in *Prunus jacquemontii* i.e. 1.33 mg/ml. Nutrition results of these five wild fruits showed that these species should be developed as nutraceuticals so that these wild edible fruits can be employed for supplementing the dietary foods of mountain people.

Key words: Wild fruits, Nutritional value, Minerals, Medicinal values, Kurram.

Introduction

Wild fruits are very important for humans which is consumed in various forms and remains very rich source of many important biological components such as proteins, carbohydrates, and crude fiber as well as providing essential minerals. Consuming these fruits in sufficient quantity and required amount, will be beneficial for people to cope with many fetal diseases and also address malnutrition (Seal, 2011; Zia-ul-haq *et al*., 2014). Among these wild fruits, an increasing interest from researchers has been noted since these fruits have a pleasant taste and also serve as one of the chief sources for medicines, nutrients, vitamins and minerals like sodium, calcium, potassium, iron, and phosphorus magnesium (Deshmukh & Waghmode, 2011; Bhagat *et al*., 2016). Wild fruits serve as an excellent source of secondary metabolites which in their own rights there are important for their medicinal value especially their antioxidant properties (Bhagat *et al*., 2016).

Millions of people in developing countries do not have enough food for their daily needs and thus, in many cases campestral communities rely upon wild resources viz., wild edible plants to fulfill their needs in a period of food scarcity. The word "wild foods" is used to represent all plant raw materials found in forests, savannah and other areas that are not cultivated but collected or gathered as the ripened form for the purpose of human consumption (Jeeva, 2009). Wild edible plants are traditionally not only used as a medicine and food source but also provide methods for their conservation as among the important world's habitats and to collect the knowledge about these plant species before this knowledge is lost forever (Abbasi *et al*., 2014).

Some wild fruits are even more important than commercial fruit and have numerous nutritional and medicinal value i.e. *Eugenia rothii* and *Terminalia citrina* have more potassium than banana and guava (Mahapatra *et al*., 2012). Similarly, the amount of calcium is also high in *Mimusops elengi* and *Phyllanthus acidus* as compared to conventional fruits such as banana and strawberry (Eromosele *et al*., 1991; Nazarudeen, 2010). Wild fruits comprise of many essential molecules viz., anthocyanin's and flavonoids which have anti-inflammatory, anticancer,

antioxidant and antimicrobial activity and are used to cure different diseases including skin and respiratory disorder, heart and bone diseases, allergy and gastro intestinal disorder in rural areas (Pachuau & Dutta, 2020). The wild edible fruits in Pakistan represent very important values in terms of a good source of energy, nutrition and medicine, all of which is of great importance and as a traditional lifestyle by tribal communities (Khan *et al*., 2015). Numerous studies have been carried out on *Rubus fruticosus* L (Rosaceae) nutritional values (Swanston -Flatt *et al*., 1990; Hummer & Janick, 2006) while few nutritional studies have been reported for *Crataegus oxyacantha* (Aslantas *et al*., 2007; Velickovic *et al*., 2016). *Nannorrhops ritchiana* and *Elaeagnus angustifolia* (Akbolat *et al*., 2008; Sheikh *et al*., 2011; Ersoy *et al*., 2013). On the other hand, no nutritional investigations have been reported for *Prunus jacquemontii.* The current study, therefore, attempts to: (i) screen five wild edible fruits for their nutritional value, (ii) develop the relationship between nutritional values and traditional uses, and (iii) reinvestigate the *R. fruticosus, C. oxyacantha,* and *E. angustifolia* nutritional values to determine the effects of soil, altitude, environment, and ecological zone on these value variations. The results of the current study will help to develop preliminary data on the nutraceutical values of these five wild edible fruits that may further be used in a dietary supplement preparation. Moreover, the investigated five edible wild fruits have medicinal and traditional uses in specifically, the Kurram region and also around the globe (Table 1).

Materials and Methods

Study area: Parachinar (Kurram) lies between 33° 53' 51 North and 70° 60° East at an elevation of about 1727m (5659 feet) above sea level (Badshah *et al*., 2016; Anwar *et al*., 2022) (Fig. 1). The climate of the Kurram region varies at different altitudes and shows differences from very hot and humid to harsh freezing conditions. The lowest temperature ever recorded in Parachinar was on 29 January 2005, when the mercury droppped to -13.4°C. The temperature on 27 June 2005 was recorded as the highiest when mercury rose to 39.9°C (Hussain *et al*., 2012). The average annual rainfall in Parachinar is 1239.9 mm, and the humidity is much highier in the morning than evening (Ajaib *et al*., 2014). Koh -e-Safaid (Spin Ghar) is the major mountain covered by snow for most of the year along with Sikaram (4728 meters) as the highiest peak (Badshah *et al*., 2016). Geographically it is located on the border of Afghanistan and Pakistan. The Kurram district is bordered in the West and North by Afghan provinces (Puktia and Nangharhar respectively), in the East it is bordered by Orakzai and the Khyber district, in the South East by the Hangu district and in the South by North Waziristan (Hussain *et al*., 2018). The total area of the Kurram ranges up to 3,380 km^2 (Muhammad *et al*., 2017).

Plant material: A total of five fresh ripened wild fruits namely, *R. fruticosus, P. jacquemontii, N. ritchiana, C. oxyacanth, E. angustifolia* (Fig. 2) were collected from different localities of the Parachinar District Kurram. A specimen of each collected fruit species is stored in a herbarium department of botany Government Postgraduate College Parachinar, Pakistan for future reference. All freshly harvested plants were immediately placed in zippered bags and each specimen was recorded with a comprehensive note in a specimen collection book to maintain proper information and labels during the collection and processing period. Information concerning these plants is illustrated in Table 1. The selection of wild fruits was done on the basis of occurrence in natural habitats, easy availability, preferred by the local people, market value and scanty information available on their nutritional and mineral potential.

However, in District Kurram, Khyber Pakhtunkhwa, Pakistan, wild plants have not been widely studied for their medicinal and nutritional value due to the area's complex geographic nature.

Preparation of sample: All samples were cleaned by rinsing in distilled water and allowed to dry at room temperature away from sunlight for several days. Dry samples were shaped and ground with a clean grinder and kept at room temperature i.e. (25-30°C) and then stored in paper bags. These were analyzed for their proximate composition and minerals analysis.

Table 1. Detail of selected wild edible fruits of the District Kurram, Pakistan. Name in parenthesis under the species name is the common name.

Name	Family	Fruit characteristics	Medicinal/traditional uses	Ref.
Rubus fruticosus auct. (L.) (Manzakhka)	Rosaceae	Fruit are juicy with sour taste when unripen and sweetish after ripened; eaten raw	Remedy for dysentery, gum inflammations and anemia	Hussain *et al*., 2018
Prunus jacquemontii Hook.f. (Arghanja)	Rosaceae	The fruit are juicy with sweet and sour taste; eaten raw	Decoction of fruit is used for cure of liver disorders and anemia and as food	Hussain *et al*., 2018
Nannorrhops ritchiana (Griff.) Atich. (Mazary)	Arecaceae	The fruit is a brown drupe	Ripened fruit are consumed as food; directly eaten as laxative, purgative and a tonic	Ali *et al*., 2020
Crataegus oxyacantha L. (Ghunza)	Rosaceae	The fruit is structurally a pome are edible, but the flavor has been compared to over-ripe apples	Fruit is used to treat gastrointestinal ailments and heart problems and consumed as food	Wang *et al*., 2013; Hussain *et al*., 2018,
Elaeagnus angustifolia L. (Sanzala)	*Elaeagnaceae*	Fruits are reddish-brown, it is consumed in dried form	The fruits are used for colic pain and as antiseptic	Hussain *et al*., 2012; Sahan *et al*., 2013

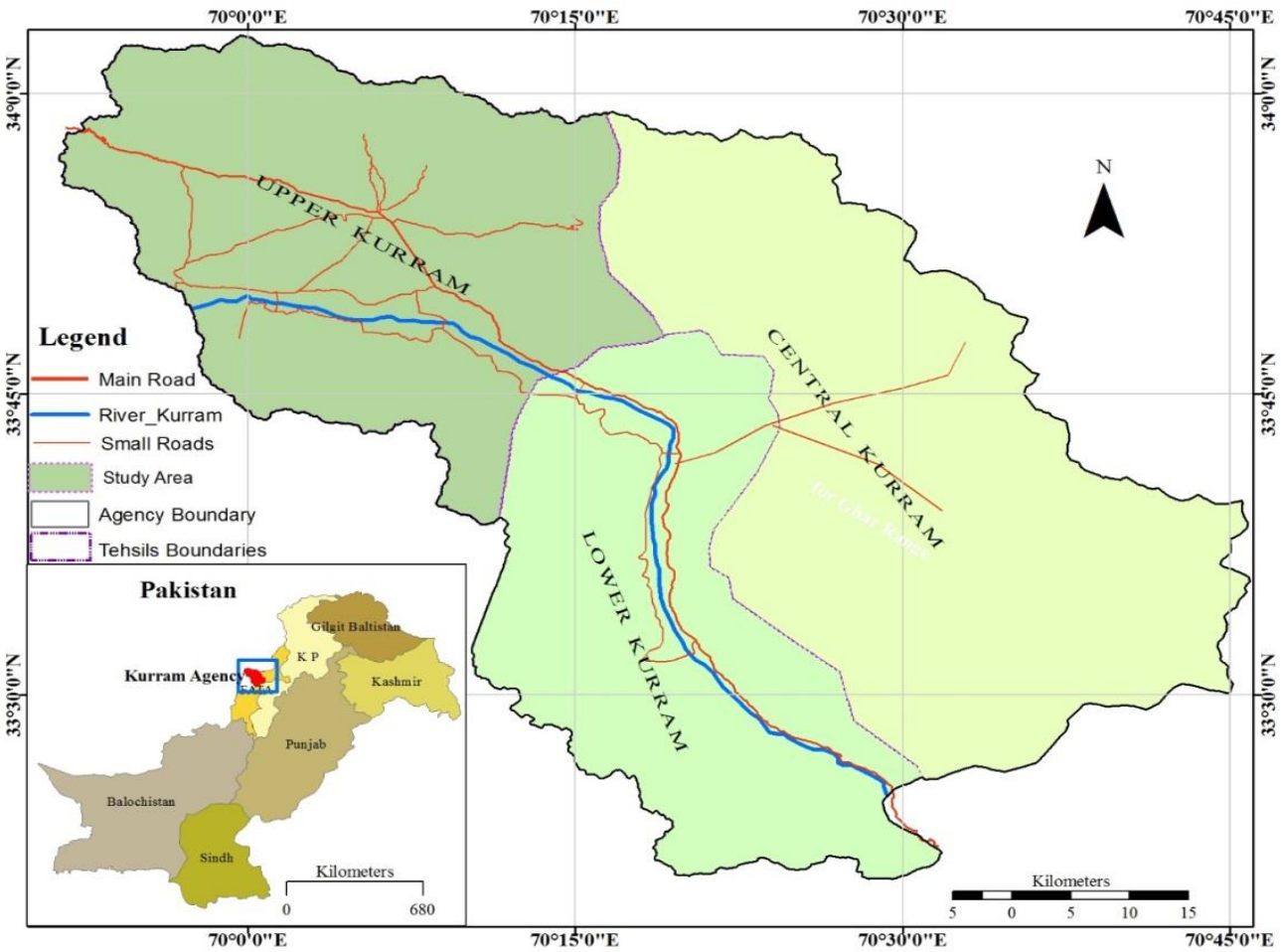

Fig. 1. Map of study area.

Proximate analysis: For their proximate composition analysis, the Association of Official Analytical Chemists (Anon., 2000 b) method was applied (Anwar *et al*., 2022). Nitrogen free extracts representing total carbohydrates, was calculated by difference. All the tests were carried out in triplicates.

Moisture determination: In order to determine the percentage moisture content, the samples were dried in an oven [PRECISION *Thelco,* model 17] at 105 ^{o} C following the AOAC method (2000) (Islary *et al*., 2016; Mih *et al*., 2017). A 1.0 g sample was placed in a dried and clean petri dish and was weighed (W1). The petri dishes were partially covered and were then placed in the oven and heated at 105 ^{o} C for 4-6 hours and placed in desiccators where they were kept for 20 minutes to cool down. They were again weighed (W2). The formula for this process is:

Moisture (%) = W1-W2 / weight of sample ×100 _____ (1)

where W1 (g) represents the early weight of petri dish with sample and W2 (g) represents later weight of petri dish after oven with sample (Batool *et al.,* 2023).

Determination of crude protein: A Kjeldhal apparatus was used for determination of N in the crude dried sample (Islary *et al*., 2016; Mih *et al*., 2017). The dried powder of the sample (1.0 g) was carefully weighed in the digestive flask. To this, 8.0 g of the digestive mixture K_2SO_4:$CuSO_4$ (7:1) and 12 ml of concentrated sulphuric acid (H_2SO_4) was added. The flask was rotated in order to mix up the contents and avoid crystal formation. The flask was heated in a steam bath and Kjeldhal apparatus was used for the distillation of the digest. During the distillation 10 ml of the NaOH (40%) was added slowly over 10 minutes to liberate the NH_3 which was collected in the form of NH_4OH. Then 20 ml of a solution of 4% Boric acid (H_3BO_3) and a few drops of methyl red indicator were added to the flask. In the process of distillation, the yellowish gradually changed into a pink color one due to the presence of NH_4OH. The distillate was then titrated against standard zero N HCL. After completion of the process, the % N was calculated as follows:

Crude protein (%) = 6.25 x %N (6.25 is protein factor for plants) __ (2)

$$\% N = \frac{(S-B) \times 0.01 \times 0.014 \times 100}{\text{Wt. of sample} \times V} \times 100$$

where S represents the sample titration reading; B represents blank titration reading; 0.01 is the normality of HCI; 100 is the dilution of the sample after digestion; V is the volume distillation; 0.014 is the milliequivalent weight of nitrogen; and 6.25 represents protein conversion factor for plants (fruits).

Determination of ash /Fixing up of Ash: For fixing up the ash Ignition method (Anon., 2000a) was used (Sajid *et al*., 2014; Czaja *et al*., 2020; Batool *et al.,* 2023). An oven dried crucible was cooled in a desiccator and weighed. 1.0 g of the dried powdered sample was placed in the crucible and was measured as its original weighed (W1). It was burned with a light fire then into a heated furnace [VULCANE 3-130]. The temperature was gradually increased to 550-600^{o}C. The sample was heated for 6 hours when it changed into gray whitish (ash) color. It was then taken out from the furnace and cooled in the desiccator and reweighed (W2). The ash was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{Wt of crucible with sample} - \text{Wt of crucible with Ash}}{\text{Sample weight}} \times 100$$

Determination of crude lipids/fat: For the purpose of determining the crude fat content a Soxhlet apparatus was used (Kassegn, 2018) with petroleum ether as solvent. In this process 1.0 g of dried powder of the sample was weighed into the Soxhlet extraction tubes. One third of the round bottom flask was filled with (100 mL) of petroleum ether with boiling point range between 40-60^{o}C. The system was then heated under reflux for five hours.

The solvent was removed under reduced pressure to leave an extracted fat in the round bottom flask. Percentage of crude fat was determined by using following formula:

Crude fat/ lipid (%) = Weight of flask with fat–weight of empty flask/ weight of sample ×100 _____ (4)

Determination of % Crude fiber: In order to determine crude fiber an acid and alkali method was adopted. An accurately determined volume (200 mL) of 2% HCL was taken in a goblet after 2.0 g of the sample was added. The mixture was boiled and stirred continuously for half an hour. It was then filtered through muslin cloth. Next, the dregs were washed with a 2% NaOH solution and a final washing with hot water gave a wet fiber and placed in a preweighed crucible which was then placed in an oven at 105^{o}C for 4 hours and cooled in desiccator. The crucible was then put in a furnace [PRECISION Thelco, model 17] at 550^{o}C for 4 hours.

Desiccator was used to cool and weight again (Islary *et al*., 2016; Batool *et al.,* 2023). Following formula was used to calculate the crude fiber.

Crude fiber (%) = Weighed of oven dried residue (W1) - Weight after ignition (W2) / Weight of sample × 100 _____ (5)

Nitrogen free extract (NFE): Nitrogen free extract was measured to show the edible carbohydrate that was the sum of the percentages of crude fat, crude protein, crude fiber and ash subtracted from 100 (Iqtidar & Saleem 2004).

NFE = 100 - % (crude fat + crude protein + crude fiber + ash) _____ (6)

Mineral analysis

Digestion of plant samples: The mineral content was determined by an acid digest of each sample according to Anon., (2000b) (Shad *et al*., 2016). About 1.0 g of each sample was mixed with 10 ml Nitric acid (HNO_3) and left standing for 12 h. The mixture was mixed with 4 ml Perchloric acid ($HCLO_4$) and boiled for 20 minutes. When the capacity of liquid in the flask decreased to 2-3 ml heating was stopped. The cooled sample was poured into a 100 ml beaker and pure water was added to the fixed level. Next, each sample was heated until the appearance of white fumes. After cooling, pure water was added to each sample. All the edible samples were filtered through Whatman No 42 filter paper into 50 ml beakers and transferred into separate plastic bottles and named accordingly.

The study of various mineral elements in these solutions was carried out using Atomic Absorption Spectrometer (Shimadzu AA-670) with suitable hollow cathode lamps. The amount of the various minerals was determined by the corresponding standard positioning curves secured by adopting standard AR grade solutions of the elements such as K, Ca, Mg, Fe, Zn, Co, Mn, Cr, and Na.

Antioxidant assays

Determination of % Free radical scavenging activity (%FRSA): The free radical scavenging activity (FRSA) of wild fruits was assessed by monitoring their ability to extinguish stable 2, 2-diphenyl 1-picrylhydrazyl hydroxyl (DPPH) free radicals (Ahmed *et al*., 2017). DPPH solution was prepared by dissolving 3.2 mg DPPH in 100 mL of 82% methanol. Then, 2800 µl of DPPH solution was added to glass vials followed by the addition of 200 µl of Crude Methanolic Extract (CME) solution in Methanol; leading to the final concentration of 100 µg/ml, 50 µg/ml, 25 µg/ml 10 µg/ml, 5 µg/ml 2 µg/ml and 1 µg/ml. Mixtures were shaken well and kept in dark at controlled room temperature (25°C-28°C) for one hour. Absorbance was measured at 517 nm by using spectrophotometer (DAD 8453, Agilent). Methanol (82%) was used as blank while mixture of 200 µl of methanol and 2800 µl of DPPH solutions were taken as negative control. Ascorbic acid was used as positive control. Each test was performed in triplicates and percentage inhibition was measured according to formula given below.

$$\% \text{ FRSA} = 1 - Ab_s / Ab_c \times 100$$

where Ab_s absorbs the test sample, while Ab_c absorbs the negative control containing DPPH and methanol instead of the sample.

Total antioxidant capacity (TAC): Phosphomolybdenum-based chlometric assay was performed to determine the total antioxidant potential and was expressed according to the microgram equivalent microgram acid per milligram of dry plant weight (g aAE / mg DW) according to (Ahmed *et al*., 2017; Jafri *et al*., 2017; Zorzai *et al.,* 2020). Each test extract contains 0.1 ml (4 mg / ml DMSO) and positive control (ascorbic acid, 4 mg / ml DMSO) to 0.9 ml reagent (0.6 M sulfuric acid, 28 mm sodium phosphate) and was mixed with 4 mm ammonium (molybdate solution in H2O). The blank contained 0.9 ml of reagent solution and 0.1 ml of DMSO without any liqueur. All tubes were placed in a water bath for 90 min at 95°C and then cooled to room temperature from where 200 µl of each sample was thoroughly transferred to the 96 well plates and each sample. The gravity was measured at 630 nm using a microplate reader (Biotech USA, Microplate Reader Alex 800). A calibration curve of ascorbic acid (y = 0.0212 × + 0.0926, R2 = 0.9913) with a final number of 100, 50, 25, 12.5, 6.25, 3.12/g/ ml was prepared and the experiment was performed in a repetitive form.

Fig. 2. Photograph of target wild edible fruits of Kurram Region Pakistan (Photographed by authors).

Statistical analysis

In order to obtain the statistical information of means value, variants and standard deviations, the R. Language software was used.

Results

Five samples of wild fruits of one kg of *R. fruticosus, P. jacquemontii, N. ritchiana, C. oxyacantha,* and *E. angustifolia* were collected and analyzed for proximate composition and minerals content (Table 2; Fig. 2). During the current study, moisture content of different fruits ranged from 11.6% to 27.1% where *R. fruticosus*, contained the lowest moisture content (11.6%) and *N. ritchieana* had the highest moisture content (27.1%). On the other hand, *P. jacquemontii, E. angustifolia,* and *C. oxyacantha* contain 26.7%, 25.1% and 19.8% moisture respectively. Moreover, crude protein was in the range of 7.9% to 11.4% in all five wild fruits. The highest content of crude protein was found in the *N. ritchieana* (11.4%) and in *P. jacquemontii* (11.3%) while the minimum protein was found in the *C. oxyacantha* (7.9%). Protein content was calculated as 9.6% in *R. fruticosus* and 8.8% in *E. angustifolia.*

The ash content was different in different wild fruits and ranged from 5.0% to 15.4% with *C. oxyacantha* comprising the minimum amount of ash (5.0%) while the maximum amount was found in *P. jacquemontii (*15.4%). On the other hand, *R. fruticosus, E. angustifolia,* and *N.*

ritchiana have 5.3%, 5.8% and 6.7% ash, respectively. Furthermore, the crude fats ranged from 0% to 11.76% in the five wild fruits. The highest amount of fat was found in *P. jacquemontii* (11.7%) and no fats were found in *E. angustifolia* and *N. ritchieana*. In addition, *C. oxyacantha* and *R. fruticosus* have 2.8% and 7.7% fats, respectively. Crude fiber value in the wild edible fruits varied among the tested species and was in the range of 0.5% to 13.4%. The lowest content (0.5%) of crude fiber was found in *C. oxyacantha* and *E. angustifolia* while highest amount was contained in *R. fruticosus* (13.4%). Other wild fruits viz., *P. jacquemontii* and *E. angustifolia* comprised 12.3% and 3.5% fiber, respectively. Moreover, the carbohydrate content in *E. angustifolia* fruits was the (85%), followed by *C. oxyacantha* (83.6%), *N. ritchieana* (77.9%), *R. fruticosus* (63.9%) and *P. jacquemontii* (49.02%).

The mineral studies of five wild fruits are depicted in Table 3 and Figure 3. The content of potassium (K) was different in the five fruits and ranged from 75.3 to 77.2 mg/L. The lowest amount of K is found in *P. jacquemontii* (75.3 mg/L) and the highest is found in *C. oxyacantha* (77.2 mg/L). The amount of potassium in *R. fruticosus*, *E. angustifolia* and *N. ritchiana* was in the range from 76.9 to 75.4%. Among the tested wild fruits, the calcium (Ca) content of different fruits was in the range from 43 to 102.7 mg/L where *P. jacquemontii* contained the least (43 mg/L) while *E. angustifolia* had the highest amount (102.7 mg/L) of Calcium. On the other hand, *R. fruticosus*, *C. oxyacantha* and *N. ritchiana* had calcium ranging from 88 to 47.3 mg/L. Moreover, magnesium (Mg) was observed in greater quantities in *R. fruticosus* (13.7 mg/L), while *E. angustifolia* contained the least (9.7 mg/L). On the other hand, the magnesium content ranged from 13.67 to 10.3 mg/L in *N. ritchiana*, *P. jacquemontii* and *C. oxyacantha* respectively.

Iron (Fe) content in wild fruits ranged from 0.6 to 3.4 mg/L and the maximum amount was found in *P. jacquemontii* (3.4 mg/l), followed by *R. fruticosus* (1.6 mg//L). The Zinc (Zn) content is different in all five wild fruits ranging from 0.3 to 0.4 mg/L. The highest amount of zinc was present in *N. ritchiana*, *P. jacquemontii*, and *R. fruticosus* (0.4 mg/L). Similarly, *R. fruticosus* has the highest level (0.3 mg/L) of cobalt (Co) while *N. ritchieana*, *C. oxyacantha*, *E. angustifolia* have same level of cobalt (0.2 mg/L). Furthermore, highest and same level (0.2 mg/L) of manganese (Mn) was found in *R. fruticosus P. jacquemontii*, and *E. angustifolia*. On the other hand, all wild fruits have the same level of chromium (Cr) (0.1 mg/L). Furthermore, *N. ritchiana* has the highest amount of sodium (Na) (6.01mg/L) followed by *P. jacquemontii* which contained (4.0 mg/L) while *R. fruticosus* contained the lowest amount (1.5 mg/L) of sodium.

The percentage of free radical scavenging activity (% FRSA) of the selected wild fruits samples, calculated by measuring the discoloration of the DPPH solution is shown in Fig. 4. The test protocol is based on the conversion of the stable purple colored DPPH radical into its diphenyl picryl yellow colored hydrazine molecule accepting electrons or hydrogen radicals from the donor antioxidant. The DPPH molecule is considered a stable free radical due to the presence of a delocalized reserve electron over the entire molecule which provides a characteristic absorption band at 517 nm. Highest free radical scavenging activity was shown by ethanol extract of *Rubus fruticosus* which was 77%, followed by *Crataegus oxyacantha* 44%, *Nannorrrhops ritchiana* 27%, *Prunus jacquemontii* 24% and *Elaeagnus angustifolia* 23%.

The total antioxidant capacity of the wild fruits estimated according to the method of (Jafri *et al*., 2017; Ahmed *et al*., 2017; Zorzai *et al.,* 20). In these wild fruits all showed a significant difference among each other in the total antioxidant capacity. Among these five wild fruits the wild fruit *Prunus jacquemontii* showed the highest value that is 1.33 mg/ml followed by wild fruit *Nannorrhops ritchiana* and wild fruit *Crataegus oxyacantha*. While the wild fruit *Rubus fruticosus* showed the lowest value (0.74 mg/ml) among all five fruits followed by wild fruit *Elaeagnus angustifolia* as shown in Fig. 5.

Table 2. Proximate composition (%) of selected wild fruits with mean value ± standard error (SE).

Plant	Moisture content (%)	Ash (% dry matter)	Crude protein (% dry matter)	Crude lipid / fat (% dry matter)	Crude fiber (% dry matter)	Carbohydrates (% dry matter)
R. fruticosus	11.6 ± 0.5	5.3 ± 0.3	9.6 ± 0.1	7.7 ± 0.1	**13.4 ± 0.1**	63.9 ± 1.1
P. jacquemontii	26.7 ± 0.3	**15.4 ± 0.3**	11.3 ± 0.2	**11.7 ± 0.2**	12.3 ± 0.2	49.02 ± 0.4
N. ritchiana	**27.1 ± 0.8**	6.7 ± 0.2	**11.4 ± 0.4**	0 ± 0	3.5 ± 0.1	77.9 ± 0.6
C. oxyacantha	19.8 ± 0.6	5.0 ± 0.1	7.9 ± 0.2	2.8 ± 0.2	0.5 ± 0.04	83.6 ± 0.3
E. angustifolia	25.1 ± 0.4	5.8 ± 0.1	8.8 ± 0.2	0 ± 0	0.5 ± 0.01	**85 ± 0.1**

Table 3. Presents Minerals composition of wild fruits (µg/g dry weight) with standard deviation.

Minerals elements	*E. angustifolia*	*R. fruticosus*	*P. jacquemontii*	*N. ritchieana*	*C. oxyacantha*
Potassium (K)	76.7 ± 0.02	76.9 ± 0.02	75.3 ± 0.03	75.4 ± 0.05	**77.2 ± 0.02**
Calcium (Ca)	**102.7 ± 0.8**	88.04 ± 0.2	43.0 ± 0.2	47.3 ± 0.1	85.4 ± 1.5
Magnesium (Mg)	9.7 ± 0.6	13.7 ± 0.8	11.5 ± 0.6	**13.67 ± 2.9**	10.3 ± 0.6
Iron (Fe)	0.6 ± 0.04	1.6 ± 0.02	**3.4 ± 0.06**	0.9 ± 0.02	0.8 ± 0.01
Zinc (Zn)	0.3 ± 0.01	0.4 ± 0.01	0.41 ± 0.01	**0.44 ± 0.01**	0.3 ± 0.02
Cobalt (Co)	0.21 ± 0.04	0.3 ± 0.1	0.08 ± 0.05	**0.23 ± 0.02**	0.2 ± 0.04
Manganese (Mn)	0.2 ± 0.004	**0.2 ± 0.01**	0.2 ± 0.01	0.1 ± 0.002	0.09 ± 0.01
Chromium (Cr)	0.1 ± 0.004	0.1 ± 0.01	0.1 ± 0.004	0.1 ± 0.003	0.1 ± 0.001
Sodium (Na)	2.4 ± 0.02	1.5 ± 0.01	4.004 ± 0.01	**6.01 ± 0.06**	2.3 ± 0.02

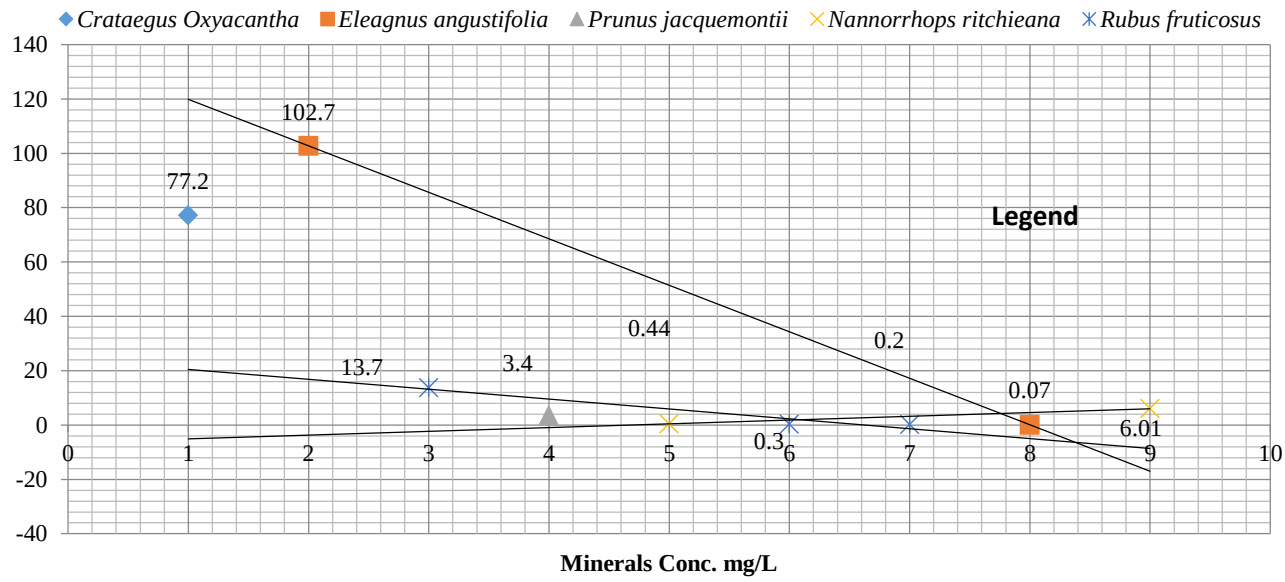

Fig. 3. Showing the maximum minerals concentrations in selected wild fruits.

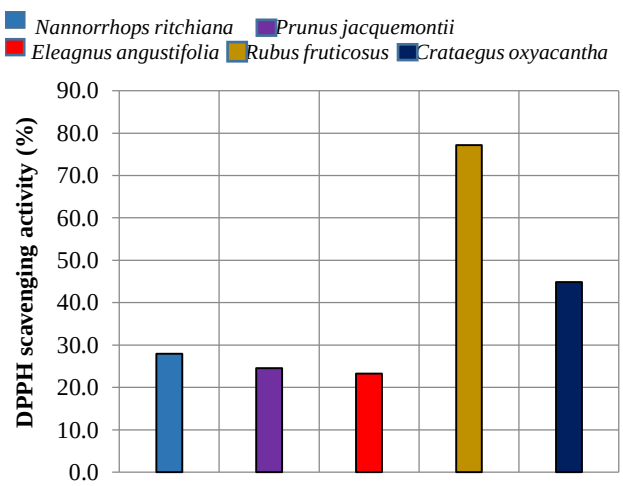

Fig. 4. Showing the % FRSA activity in selected wild fruits.

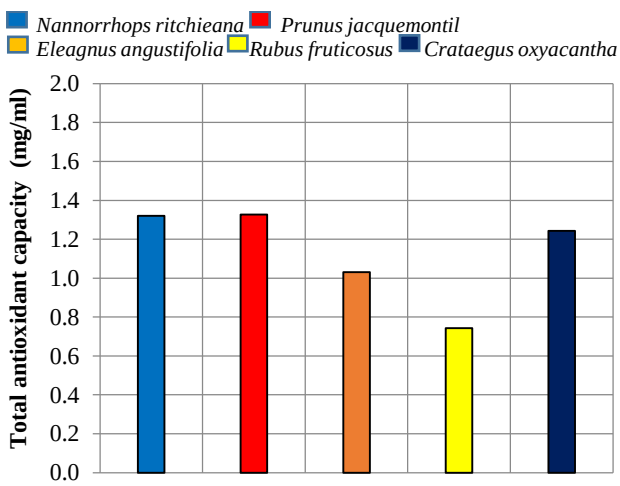

Fig. 5. Showing the total antioxidant activity in selected wild fruits.

Discussion

This research was designed to document the nutritional values of traditionally important wild fruits, their edibility and promoting the awareness of this to the people of the study area. The present research results illustrate that these are nutritionally rich and relatively safe for consumption. Current results showed that *R. fruticosus, P. jacquemontii, N. ritchiana, C. oxyacantha* and *E. angustifolia* are important food supplements for the local communities. The proximate analysis of the five wild fruits shows that the moisture content was generally high especially for *N. ritchiana, P. jacquemontii,* and *E. angustifolia* with 27.1%, 26.7% and 25.1% respectively. Moreover, Sahan *et al*., (2015) reported a 20% moisture content in unpeeled *E. angustifolia* fruit. We however, found higher moisture content compared to the reported value in previous studies which might be because of developing periods of the fruit or ecological conditions (Falahi *et al*., 2020). Calisir *et al*., (2005) in their work on the wild plum species of *Prunus* in Turkey reported a lower value of moisture than our findings. A high moisture content is required to maintain the turgidity of cells, their freshness and it is considered that 20% of total water must come from the food moisture. On the other hand, the lower the water content the slower the degradation of fruits by microorganism becomes and thus these can be more easily preserved (Khan *et al*., 2013; Mironeasa *et al*., 2016; Mih *et al*., 2017; Ullah *et al*., 2017; Rusmadi *et al*., 2020).

In current study illustrated that the maximum amount of crude protein is present in *N. ritchiana* 11.4 %) and in *P. jacquemontii* (11.3%) and the minimum was found in *C. oxyacantha* (7.9%). The protein amount in the fruits of *C. oxyacantha* was lower than those reported by other researchers (Khan *et al*., 2013; Mironeasa *et al*., 2016). On the other hand, *C. oxyacantha* seeds (13.5%) and leaves (21.8%) have a higher crude protein than the fruits (Bukhsh *et al*., 2007). Culturally, the above mentioned wild fruits are the important part of our daily diet. Our study showed that these fruits are a good source of protein and their consumption would provide energy to overcome malnutrition common in rural areas. According to World Health Organization (WHO) the recommended dietary

allowance (RDA) of protein, for children, an adult male and adult female is 28, 63 and 50 g respectively. In Pakistan the average protein intake is 43.4 g/day (Ullah *et al*., 2017). Protein is considered as an important component of body tissues after water and is necessary for growth (Rehman & Adnan., 2018) because many illnesses can arise due to insufficient intakes of protein like organ failure, weakness of muscles, impaired mental health, and edema (Rusmadi *et al*., 2020). This study demonstrated that the tested wild fruits represent a rich source of protein and are thus good in the daily diet for humans. Proteins perform various functions in the form of enzymes, transport carriers in membranes, as hormones, and help in growth, maintaining and repairing the body (Ullah *et al*., 2017; Bvenura & Sivakumar, 2017).

The selected wild fruits were also rich in ash content. For instance, 15.4% is recorded for *P. jacquemontii* which is lower than that by Calisir *et al*., (2005) who reported 28% ash in a wild plum species in Turkey. Fruits contain the maximum amount of ash and are also expected to have maximum amount of minerals which helps in growth and development when eaten (Rusmadi *et al*., 2020). Ash is defined as the fireproof and non-living part of fuel left after burning. It is considered as very important as it contains both micro and macro nutrients like calcium, sodium, potassium, and zinc which help with the psychological function of the body (Khan *et al*., 2013; Ullah *et al*., 2017; Rehman & Adnan, 2018). Current results showed that *P. jacquemontii* (11.7%) and *R. fruticosus* (7.7%) are a good source of crude fat which means that they are the main source of fats (energy). Calisir *et al*., (2005) determined only 1% fats in the wild plum (*Prunus* sp) Turkey species which is lower than the total content of fat in our study. A diet which provides about 1-2% of caloric energy in the form of fat is enough for people to consume (Khan *et al*., 2013; Rehman & Adnan,, 2018). Moreover, no fat is present in *N. ritchiana* and *E. angustifolia* and the lower the value of fat in fruits is, may helpful in weight loss diets (Rusmadi *et al*., 2020).

The crude fiber was recorded highest (13.4%) in *R. fruticosus,* (12.3%) in *P. jacquemontii* and lowest for *E. angustifolia* (0.5%). The fiber intake is recommended for adults to be 25 g per day (Bvenura & Sivakumar, 2017). It has been reported that fiber helps in prevention of overweight and constipation. Dietary fiber as indigestible carbohydrates and lignin that is native and an integral in plants. It also helps in removing the carcinogens from the body and prevents the absorption of extra cholesterol. It thus cleanses the digestive tract. It also works against hypercholesterolemia and diabetes mellitus by preventing the consumption of excess foods rich in starch (Khan *et al*., 2013; Sahan *et al*., 2015; Mih *et al*., 2017; Ullah *et al*., 2017; Rusmadi *et al*., 2020). Moreover, three investigated wild fruits have a high concentration of carbohydrate viz., *E. angustifolia* (85%), *C. oxyacantha* (83.6%) and *N. ritchiana* (77.9%). Carbohydrates are the chief source of energy. The average intake of carbohydrate is 349 g per day as reported in Pakistan (Khan *et al*., 2013; Ullah *et al*., 2017; Rehman & Adnan, 2018). Fruits that contain good amounts of carbohydrates will be suitable for diabetes and hypertensive patients requiring minimum sugar diets (Khan *et al*., 2013; Ullah *et al*., 2017; Rusmadi *et al*., 2020).

These fruits are also rich in both macro and microelements which are very important in the human diet. The results show that these fruits are good sources of K, Mg, Ca, Fe, Zn, Mn, Cr, Co and Na. Potassium was calculated maximum 77.2 mg/L in *C. oxyacantha*. Potassium is one of the most important minerals in the body. It helps regulate fluid balance, controls skeletal muscle contraction and nerve signals (Mih *et al*., 2017). Notably, all five wild fruits have reasonable amount of potassium. The current study showed 77.2 mg/L for potassium in *C. oxyacantha* while Han *et al*., (2012) reported 13,531.9 ppm of potassium in *C. oxyacantha*. Calcium concentration in *E. angustifolia* was recorded as a maximum (102.7 mg/L) and it is the one of the most important minerals for the human body. Among the tested wild fruits, the results reveal that these are a good source of calcium which is associated with bone, teeth, muscle and heart functions (Mih *et al*., 2017; Bvenura & Sivakumar, 2017). Ersoy *et al*., (2013) carried out research on *Elaeagnus* fruits in Turkey and calculated an amount of 547.6 ppm calcium.

R. fruticosus and N. ritchiana both comprise of a good amount of Mg viz., 13.7 mg/L and 13.6 mg/L respectively and the amount of Mg in *R. fruticosus* in our study was less than the amount obtained from blackberry (*R. fruticosus;* 83.4–381.2 mg/L) wine by Klaric *et al*., (2011). Naseem *et al*., (2005) recorded 15.7% of Magnesium in *N. ritchiana* which is slightly higher than our results. This may be as a result of environmental factors like weather, sunlight intensity, and soil composition which all affect plant growth (Falahi *et al*., 2020). Magnesium plays a vital role in body metabolism and reproduction (Ullah *et al*., 2017) and acts as a co-factor for more than 300 enzyme systems and protein and nucleic acid synthesis in the body (Ullah *et al*., 2017; Mih *et al*., 2017). It helps to maintain normal nerves and muscle function, support a healthy immune system, keep the heartbeat steady, and plays an important role in reproduction as well as bone growth (Al-Fartusie & Mohssan, 2017).

Among the wild fruits of this study, *P. jacquemontii* and *R. fruticosus* contain appreciable amount of iron viz., 3.4 mg/L and 1.6 mg/L respectively. According to previous studies by Calisir *et al*., (2005) the wild plum species of *Prunus* contain 4.9 % iron, slightly higher than our tested *P. jacquemontii*. On the other hand, previous investigations showed that blackberry fruits had a total Fe of 0.62 mg, which was slightly less than the amount of Fe in our study (Velciov *et al*., 2019). Our results are however, in line with the results of Klaric *et al*., (2011) who reported the total Fe content in blackberry fruit wine being between 0.1-2.1 mg/L. Iron is an important component of hemoglobin, the substance in red blood cells that carries oxygen from the lungs throughout the body and for normal functioning of CNS (Al-Fartusie & Mohssan, 2017; Mih *et al*., 2017). In addition, iron is essential for various biological responses such as gene regulation, cell growth and differentiation, oxidation reduction reactions, and in the electron transport system (Ullah *et al*., 2017, Ogunyinka *et al*., 2017).

Wild fruits are also a good potential source of Zn which is present in the greatest amount in *N. ritchiana* (0.44 mg/L) and *P. jacquemontii* (0.4 mg/L). A high Zinc content is essential for the normal functioning of the immune system and healthy skin (Naseem *et al*., 2005). In addition, it helps in the growth of stunted children and also prevents children from getting intestinal diseases (Ullah *et al*., 2017). Traditionally, *N. ritchiana* is used as a laxative (Hussain *et al.,* 2018) and the presence zinc therefore supports the traditional uses. Cobalt concentration was recorded as 0.3 mg/L in *R. fruticosus* and is lower than that reported previously by Klaric *et al*., (2011) and Zia-Ul-Haq *et al*., (2014) from *R. fruticosus.*

Manganese was present in three of the wild fruits in the same concentration (0.2 mg/L) including *R. fruticosus*, *P. jacquemontii* and *E. angustifolia*. Ersoy *et al*., (2013) reported 4.6 ppm manganese in *Elaeagnus* fruits and Sahan *et al*., (2013) analyzed for an amount of 4.51 mg of magnesium per kilogram (kg) in *E. angustifolia*. Chromium is also present in all five wild fruits in the same concentration (0.1 mg/L) while Ersoy *et al*., (2013) recorded an 0.2 ppm content of chromium in *Elaeagnus* fruits. The highest amount of sodium was present in *N. ritchiana* (6.0 mg/L) followed by *P. jacquemontii* (4.0 mg/L). Sodium is an important electrolyte which maintains the water balance around the cell and controls blood pressure (Mih *et al*., 2017).

The results of FRSA and TAC was significant in wild fruits. The free radical scavenging activity was reported high in *R. fruticosus* 77%, while Zafra-Rojas *et al*., (2018) and Zozri *et al*., (2020) calculated 13656.27 ± 532.66 (µmol TE/100 g db), 0.28 e mg/mL DPPH activity in *R. fruticosus* (blackberry) respectively. In our research we found DPPH activity in *Crataegus oxyacantha* 44% while Kostic *et al*., (2012) studied the DPPH scavenging activity of *C. oxyacantha* fruits and calculated high antioxidant activity with DPPH radical transformation value 89.9% in the methanol-water (50/50, v/v%)) extract. Benabderrahmane *et al*., (2021) reported DPPH radical scavenging activity ranged from 0.183 and 0.098 mM TRE, in the extract of *C. oxyacantha* fruits. *Nannorrhops ritchiana* showed 27% and *Elaeagnus angustifolia* 23% DPPH activity. Essa *et al*., (2018) calculated antioxidant activity of *N. ritchiana* with SC50 39.4 ± 1.06 µg/ml in comparison with ascorbic acid (SC50 1.8 ± 0.35 µg/ml) and Cansev *et al*., (2011) reported Free radical scavenging activity for Mesocarp of *Elaeagnus angustifolia* 28.03 g of dried mass water extracts equal to µmol Throlox.

Antioxidants are self-made or natural substances that can stop or delay certain types of cell damage and are found in many fruits and vegetables. The main function of antioxidants is to prevent oxidation in various contexts, by the use of antioxidants, human body is protected from oxidative stress, cardiovascular, neurological and carcinogenic diseases, like cataracts, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract (Yadav *et al*., 2016).

Conclusion

During the current study, five wild edible fruits viz., *R. fruticosus, P. jacquemontii, N. ritchiana, C. oxyacantha,* and *E. angustifolia* of Kurram region of Pakistan were selected for study based on their local uses. From the current results it was concluded that these wild fruits are not only delicious and refreshing but also a rich source of nutrients like proteins, fats, carbohydrates and minerals like Potassium, Calcium, Magnesium, Iron, Zinc, Cobalt, Manganese, Chromium and Sodium as well as antioxidant potential. Our results showed that consumption of the investigated wild fruits could supplement the nutritional requirement of the Kurram people. In addition, these wild fruits could play an important role in the fledging nutraceutical industry due to their high concentration of nutritional elements. In addition, the production of these wild plants in the forest on a large scale should be encouraged in order to provide sufficient renewable sources of these valuable edible plants.

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