Volume 8, Issue 3, 2018, 3178 - 3186 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 25.04.2018 / Revised: 19.05.2018 / Accepted: 10.06.2018 / Published on-line: 15.06.2018

Assessment of phenolic contents, essential/toxic metals and antioxidant capacity of fruits of *Viburnum foetens* decne

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ABSTRACT

Since antiquity, edible wild fruits played an imperative role in supplementing the diet of millions of rural inhabitants around the globe. The present study was designed to evaluate the polyphenols, essential/toxic trace metal contents, proximate composition and free radical scavenging capacity of the edible wild fruit of *Viburnum foetens* consumed by the inhabitants of Himalayan region-Pakistan. Fruit samples were collected from different localities of the Himalayas. Proximate composition and the metal levels were estimated using standard (AOAC) methods and atomic absorption spectrometry. Water and acetone extracts were analyzed to estimate the phenolics and ascorbic acid contents. DPPH, hydroxyl and hydrogen peroxide radicals scavenging, ferrous ion chelating, ferric ion reducing antioxidant power (FRAP) and phosomolybdenium complex assays were conducted to determine the antioxidant capacity. The fruit samples showed significant fibers, fats and proteins contents along with essential metals. Flavonoids were highest in concentration (113.92 ± 1.46 mg Rt/100 g, FW) followed by flavonols and phenolics. Acetone extract exhibited the highest level of total antioxidant capacity (84.67 ± 0.48 µM AAE/100 g, FW) and DPPH radical scavenging capacity (84.62 ± 0.63%), followed by percentage hydroxyl radical scavenging at 75.53 ± 0.95. Significant correlations among the phenolics, ascorbic acid and metals contents with free radicals scavenging capacity were found. The present study revealed that edible wild fruits of *V. foetens* are rich in nutrients and health beneficial natural antioxidants and possess significant antioxidant potential to scavenge the free radicals. It indicated that wild edible fruits could be used as potential functional food or value-added ingredients to promote consumers health.

Keywords: *Himalayas; Viburnum foetens; metals; polyphenols; antioxidant; Pakistan*

1. INTRODUCTION

Traditional knowledge and aboriginal evidence propose that a variety of wild edible plant species in the Himalayan region have played an important role in providing food and medicine for human beings and animals as well [1]. The utilization of wild edible fruits, as a diet supplement in times of plenty and as one of the major coping mechanisms at times of food shortage and famine has been recognized extensively [2,3]. Wild fruits offer vitamins, flavoring agents, and compounds of nutritional, gastronomic and social importance derived from secondary metabolism [4]. Composition analysis of edible wild fruits and vegetables plays a fundamental role to evaluate their nutritional significance [5,6]. Furthermore, households harvesting of wild fruits can boost rural employment and generate income through processing, which makes them exceptionally important and therefore adding value [7].

Reactive oxygen species (ROS) including superoxide anion, hydroxyl radical, singlet oxygen, hydrogen peroxide and other oxidants are key factors in disease pathology and concerned with many sensitive and persistent health disorders, such as cancer, diabetes, atherosclerosis, aging, immune suppression and neurodegeneration in human beings [8]. The human body has an intrinsic anti-oxidative mechanism, which initiates anti-mutagenic, anti-carcinogenic, anti-aging responses, and others related biological functions [9,10]. Antioxidants balanced or neutralize free radicals and have been inversely associated with morbidity and mortality caused by degenerative disorders. Various investigations on fruits, vegetables, grain and medicinal herbs have indicated the occurrence of antioxidants such as phenolics, flavonoids, ascorbic acid, tannins and proanthocyanidins [9,11-13]. Interestingly, use of naturally occurring antioxidants has significantly been augmented in food, cosmetic and pharmaceutical products, because of their free radicals scavenging capability and multi affectedness in multitude and level of activity [14]. There is little known about the active constituents of plants, which are associated with the reduction of chronic diseases, but antioxidants appear to play a major role in the protective effect of plant-based medicines [15] and search for novel and natural antioxidants have ever since increased.

Viburnum, a genus of family Caprifoliaceae, comprises about 200 species disseminated in temperate and subtropical regions of Asia, North America, Bhutan, Tibet, and Malaysia. In Pakistan, it is represented by 6 species. *Viburnum foetens* Decne is a large deciduous shrub (Figure 1), found at 4000-8000 ft elevation in different localities including Murree, Gilyat, Abbottabad, Muzafarabad, Kaghan of the Himalayan region-Pakistan. Fruits of *V. foetens* are edible, sweetish, black when ripe, drupe, compressed one seeded and appeared in July-September. Medicinally, *V. foetens* fruits are sedative, purgative and blood purifier [16,17]. Furthermore, this plant is antidiabetic [18] and antibacterial [19]. Young shoots of *V. foetens* are used as tooth brush (Miswak) and leaves as fodder and forage for livestock. Although fruits of *V. foetens* are edible and used in folk recipes to treat various diseases but little is known about its nutraceutical aspects. The present study was aimed to measure the nutritional composition, total phenolics, selected metals and *in vitro* antioxidant capacity of the edible fruits of *V. foetens*. Inter-

relationships among the measured variables would also be assessed in the fruit samples. It is anticipated that the present study would provide valuable information related to the health benefit associated with the wild fruits which could be potential functional food to improve the consumers' health.

2. EXPERIMENTAL

2.1. Cultural significance. Cultural value of *V. foetens* was estimated using the method as explained earlier [20]. Data on medicinal and ethnobotanical aspects were collected by semi-structured interviews with local informant from four major sites of lower Himalayas including Abbottabad, Haripur, Mansehra, and Murree Hills. Cultural importance index was deliberated using formula as under:

$$CI = \sum_{i=1}^{i=NU} \frac{URi}{N}$$

where, N is the number of informants and UR is the use report in each use category.

2.2. Sampling. Fresh fruits were collected during field visit; the samples were washed carefully with tap water followed by deionised water and desiccated at room temperature. Samples were dried at 55°C for 24 hours in the electric oven (MEMMERT N-12880 KI, Germany) [21], then grinded with a porcelain pestle and mortar. The fine powder was sieved through a muslin cloth and kept in desiccators for further analysis.

Figure 1. Edible wild fruits of *Viburnum foetens*.

2.3. Proximate analysis. Moisture, crude proteins, crude fats, carbohydrates, crude fibers and ash contents were estimated by AOAC methods [22]. The crude protein contents were calculated by the macro-Kjeldahl method; crude fats by petroleum ether through Soxhlet extractor, ash content by ignition at 600 ± 15 ºC; crude fibers by acid-base digestion; carbohydrates by difference method (100 - (g moisture + g protein + g fat + g ash), and calorific value was estimated by following equation:

Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid).

2.4. Analysis of essential/toxic metals. For the determination of essential/toxic metals, about one gram (~1.0 g) of fruit sample was digested in a blend of nitric acid and perchloric acid at 80-85°C until a clear solution was obtained [23,24]. A blank was also prepared in the same way. Selected essential and toxic trace metals including Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Pb, Sr and Zn were quantified using atomic absorption spectrophotometer (Shimadzu AA-670, Japan), employing the calibration line method under optimum analytical circumstances. The reagents and standard solutions used were of AAS grade (>99.99% purity). The accuracy of the method was evaluated using certified reference materials (NIST-SRM 1515) and the recovery was in the range of 97 to 104% for all the metals.

2.5. Estimation of total phenolics, flavonoids and flavonols contents. Two step extraction procedure i.e. hydrophilic (aqueous) and hydrophobic (acetone) was adopted as described before [25]. Briefly, 1.0 g powered sample in triplicate was mixed with 10 mL of deionised water and centrifuged at 6000 rpm for 15 min. The supernatant was collected in a clean flask. This procedure was repeated thrice and supernatants were pooled in a flask. The solid residue was re-extracted 3 times in acetone (1:10 w/v) as mentioned earlier and supernatants were also pooled.

The total phenolic contents (TPC) were calculated following the method as described before [26]. In brief 1.0 mL aliquots of water and acetone extracts were mixed with 5 mL of 10 fold diluted Folin-ciocalteu reagent followed by the addition of 4 mL sodium carbonate (7.5%). The whole mixture was allowed to stand for 90 minutes at room temperature before recording the absorbance at 760 nm. Final values were expressed as mg gallic acid equivalents in 100 g of fresh sample (mg GAE/100 g, FW). Data were presented as mean ± SD for each triplicate measurement.

Total flavonoids contents (TFC) were determined using the modified colorimetric method as described earlier [26]. Briefly, 5 mL of each water and acetone extract in triplicate was transferred to the test tubes followed by the addition of 0.3 mL sodium nitrite (5%) and mixed for 5 min. Furthermore, 0.3 mL of 10% aluminium chloride was added into it and after 6 min, 2 mL sodium hydroxide was added to stop the reaction. The mixture was diluted with distilled water up to 10 mL and the absorbance was immediately measured at 510 nm. Measured values of flavonoids content were articulated as mg rutin equivalents in 100 g of fresh sample (mg Rt/100 g, FW) and data were expressed as mean ± SD for each triplicate measurement.

Total flavonols contents (TFlC) were estimated using the reported method [27]. Briefly, 2.0 mL of aluminium trichloride (2%) and 3 mL sodium acetate (50 g/L) solutions were added in 2.0 mL of extracts in triplicates. Samples were kept at 20°C for 2.5 h before recording the absorption at 440 nm. Measured levels of flavonols contents were expressed as mg rutin equivalents in 100 g on fresh weight basis (mg Rt/100 g, FW) and data were presented as mean ± SD for each triplicate measurement.

2.6. Determination of ascorbic acid content. Ascorbic acid content (AAC) in the fruit of *V. foetens* was deliberated following the scheme as described previously [28]. Dried extracts of the samples in triplicates were re-extracted with meta-phosphoric acid (1%, 10 mL) for 45 min at room temperature and filtered. The

filtrate (1.0 mL) was mixed with 9 mL of 2, 6-dichloroindophenol (0.8 g/1000 mL) and the absorbance was measured within 30 minutes at 515 nm. Ascorbic acid contents were calculated on the basis of calibration curve of L-ascorbic acid (0.006-0.1 mg/mL; y = 3.006x + 0.007; R^2 = 0.999). Final values were expressed as mg ascorbic acid equivalents in 100 g on fresh weight basis (mg AA/100 g FW). Data were presented as mean ± SD for each triplicate measurement.

2.7. Free radicals scavenging activity

2.7.1. DPPH radical scavenging activity. DPPH scavenging activity in the aqueous and acetone extracts was determined according to previously reported method [29]. Briefly, 2.0 mL of each extract and standards were added to the 5 mL of DPPH solution (0.1 mM in methanol) and vortexes vigorously followed by incubation in dark for 30 minutes at room temperature. The decolourization of DPPH was measured against blank at 517 nm and percentage inhibition was calculated using the following relationship:

$$\%Inhibition = \frac{(A_{Blank} - A_{Sample})}{(A_{Blank})} \times 100$$

2.7.2. Hydroxyl radical scavenging activity. Hydroxyl radical scavenging capacity of fruit extracts was estimated following the method [30], which is based on Fenton reaction. In short, 2.0 mL of 0.2 M phosphate buffer (pH 7.2), 0.04 mL ferrous sulphate (0.02 M), 2 mL of extract and 1 mL of 1, 10-phenanthroline (0.04 M) were mixed, followed by the addition of 0.1 mL of 7 mM H_2O_2 to start the Fenton reaction. Whole mixture was incubated for 5 min at room temperature and the absorbance was measured at 560 nm. Hydroxyl radical scavenging activity in percentage was calculated as:

$$Scavenging.Activity(\%) = \frac{(A_{Blank} - A_{Sample})}{(A_{Blank})} \times 100$$

2.7.3. Hydrogen peroxide scavenging activity. Hydrogen peroxide radical scavenging activity was determined as explained before [31]. Precisely, 4 mL of each water and acetone extract in triplicate was mixed with 2.4 mL of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M, pH 7.4) and incubated for 10 min at room temperature. The absorbance was measured at 230 nm against blank without H_2O_2, and percentage scavenging activity was determined as follow:

$$Scavenging.Activity(\%) = \frac{(A_{Blank} - A_{Sample})}{(A_{Blank})} \times 100$$

2.7.4. Ferrous ion chelating activity. The ferrous ion chelating ability was estimated by the method of [32]. In short, 2.0 mL of each extract in triplicate was added to 2.0 mL of ferrous sulphate (0.125 mM), and the reaction was started by the addition of 2 mL of 0.3125 mM ferrozine. The mixture was shaken vigorously and left standing at room temperature for ten minutes and absorbance was measured at 562 nm against blank prepared in the same way using ferrous chloride and water. EDTA (0.625-5.0 mg) was used as positive control and sample without extract or EDTA served as negative control, final results were expressed in the percentage inhibition of ferrozine-Fe (II) complex as follow:

$$Chelating.Activity(\%) = \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \times 100$$

2.7.5. Ferric ion reducing antioxidant power (FRAP). The ferric ion reducing antioxidant power of fruit extracts was estimated following the method described before [33] with slight modification. Briefly, 2.0 mL of extract in triplicate sample was mixed with 2.0 mL of phosphate buffer (0.2 M, pH 6.6) and same volume 0.1% potassium ferricyanide. Mixtures were incubated at 50°C in water bath for 20 min and 2 mL of trichloroacetic acid (10%) was added to stop the reaction. The upper portion of solution (2 mL) was mixed with distilled water of 0.01% ferric chloride (2 mL of each) and kept at room temperature for 20 min, and absorbance was measured at 700 nm against blank. A higher of absorbance of reaction mixture revealed greater reducing power. Gallic acid was used as positive control and final values were expressed as the concentration of antioxidant having ferric reducing ability in 100 g of fresh sample (µM GAE/100 g, FW). Data were presented as mean ± SD for triplicate measurements.

2.7.6. Phosomolybdenium complex assay. Total antioxidant capacity (TAC) of the studied samples was deliberated using Phosomolybdenium complex assay (PCA) as described earlier [34]. Precisely, 2.0 mL of each extract in triplicate was added to 6.6 mL of reagent mixture (0.6 mol/L sulphuric acid, 28 mol/L sodium phosphate, and 4 mol/L ammonium molybdate), and incubate at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against blank. Fine results were expressed as relative antioxidant activity (RAA) compared to ascorbic acid in 100 g on fresh weight basis (µM AAE/100 g, FW) and data were presented as mean ± SD for triplicate measurements.

2.8. Statistical analysis. Statistical analyses were performed using SPSS software 13.0 (SPSS Inc., Chicago, IL, USA). Results were subjected to ANOVA, and differences among means were located using Tukey's multiple comparison tests. A *p*-value less than 0.05 ($p < 0.05$) was regarded as statistically significant. Basic statistical parameters and correlation coefficients among the measured variables were also calculated. All data were reported as the mean ± SD for three to five replicates.

3. RESULTS AND DISCUSSION

3.1. Ethno-medicinal and cultural importance. Local residents in the Himalayan region of Pakistan use *V. foetens* for various purpose such ripened fruits of this species are edible eaten raw, and considered good to treat constipation. Ethnobotanically, branches of this species are used as tooth brush and in making baskets. These findings were in agreement with previous reports [17]. Cultural importance index (CI) and mean cultural index (mCI) explain cultural significance and comparison of plant knowledge in different cultures, and also showed intercultural variations [35]. As illustrated in Figure 2, maximum cultural index of *V. foetens* was calculated for Abbottabad (0.721), followed by Murree and Mansehra (0.637 and 0.537). As *V. foetens* is a commonly growing species in Abbottabad and its surrounding areas, and local people are familiar with its ethnobotanical, medicinal and food values. Such aspects support the high cultural index of this species in this area.

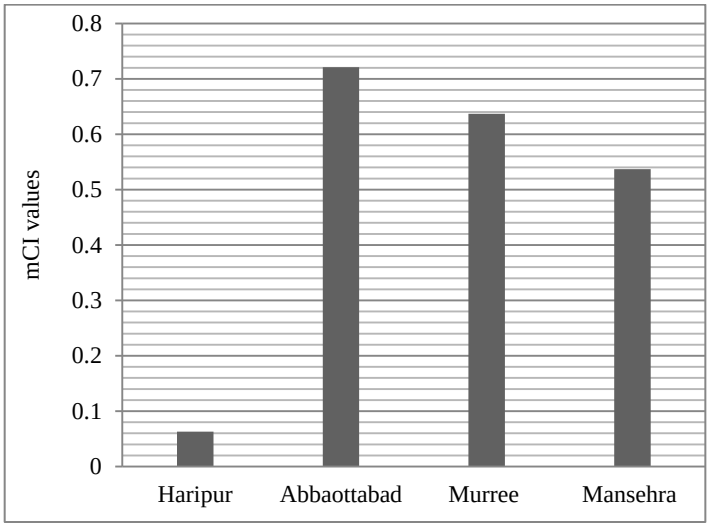

Figure 2. Mean cultural index (mCI) of *V. foetens.*

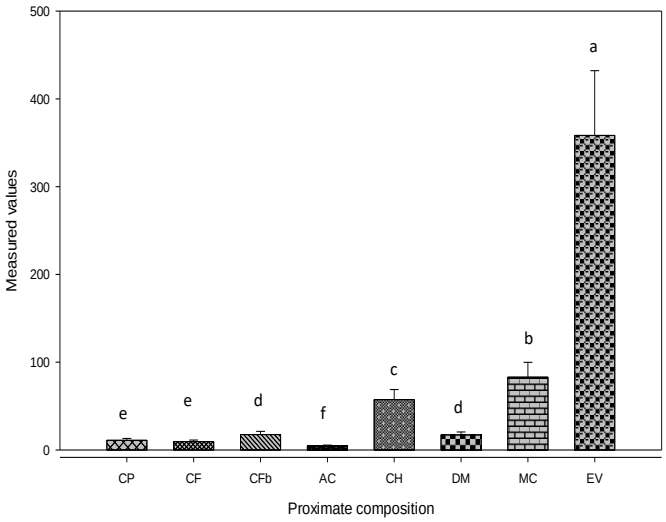

Figure 3. Proximate composition of *V. foetens* fruit. [CP-crude proteins (%), CF-crude fats (%), CFb-crude fibers (%), CH-carbohydrates (%), DM-dry matter (%), MC-moisture content (%), EV-energy value (kcal/100g). Each bar shows mean of 3-5 replicates ± SD. Letters (a-f) indicates significant difference at $p<0.05$].

3.2. Proximate composition. Physical properties of fruits are known to vary with their moisture contents [36]. Dry matter and moisture contents were determined on a fresh weight basis, while all other parameters were based on dry weight. As shown in Figure 3, the moisture content of *V. foetens* fruit was comparable to conventional fruits that ranges between75-95% [37]. Dry matter content was in the range but ash content was comparatively lower than *Zizyphus mauritiana, Strychnos innocua* and *Strychnos spinosa* reported from Malawi state India [38]. In edible wild fruit of *V. foetens* carbohydrates content was maximum at 57.31%, followed by crude fibers, proteins, fats and ash contents (17.61%, 10.96%, 9.478% and 4.623%, respectively) with significant difference ($p<0.05$). Crude proteins and fats contents were significantly higher than reported levels in the fruits of baobab, orange, mango, grapes, banana, papaya, *A. digitata, S. birrea, S. spinosa* and *Vanguenia infausta* from Botswana [39-41]. The tradition of chewing fruits or eat them as snacks seems appropriate for the particular purpose of satisfying hunger in view of their carbohydrates content and energetic value [42]. Fruits of *V. foetens* were rich in crude fibers content, while carbohydrates value was lower than reported earlier for other fruits [38,41,43]. The high energy value is mainly contributed to the fat content of the fruits. Our results indicated that Consumption of 100g of *V. foetens* fruits provides 358.4 kcal/100g of calorific value.

3.3. Essential/toxic metal levels. As shown in Figure 4, a total of 14 essential and toxic trace metals (Ca, Mg, K, Na, Fe, Cu, Mn, Sr, Zn, Li, Cr, Co, Cd and Pb) were analyzed in the present study. Potassium, calcium, magnesium, and sodium are essential in the human metabolism, proper functioning of cells, tissues, bones and muscular health, and to maintain nervous and immune systems [43-44]. The biological activities of Cu, Fe, Zn and Mn, are strongly associated with the presence of unpaired electrons that allow their participation in redox reactions. It is assumed that these trace metals such as iron, zinc and manganese play a key role in the protection mechanisms by scavenging free radicals [37]. In the studied sample measured concentration for K was highest at (901.9 mg/kg, FW), followed by Ca and Mg (315.5 and 86.51 mg/kg, FW), whereas Cr exhibited lowest value (0.055 mg/kg, FW) significantly different at $p<0.05$. Concentrations of K, Ca, Mg and Na in the fruit of *V. foetens* were relatively higher than guava, pear, orange, apple, banana and different wild fruits [39,45]. As regard to micro-nutrients, Fe was the main element with the highest concentration at 11.73 mg/kg, FW ($p<0.05$), while rest of the metals were in following order: Sr > Zn > Mn > Cu showing no significant difference ($p<0.05$). However, measured levels of these elements were comparatively higher than previous reports in guava, pear, orange, apple and banana [39,40].

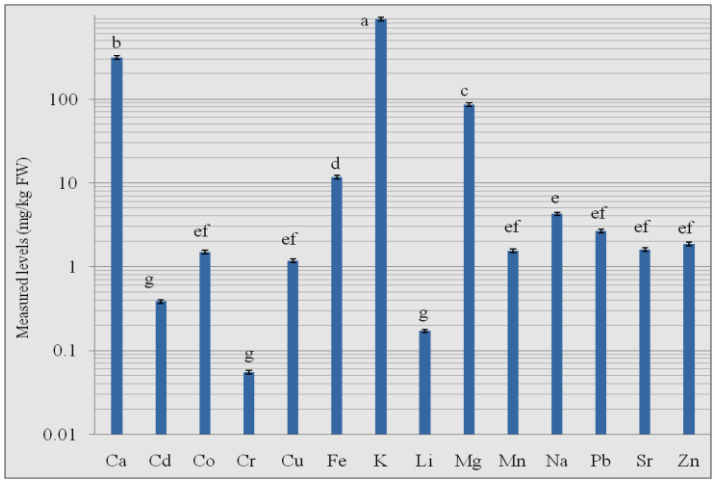

Figure 4. Essential/Toxic metal levels in *V. fotenes* fruit (mg/kg) on fresh weight basis. Each bar shows mean of 3-5 replicates ± SD. Letters (a-g) indicates significant difference at $p<0.05$]

Some elements which may contribute to biological processes, but have not been established as essential such as lithium, cadmium, chromium, cadmium and lead, and are best known for their toxicological properties [46]. Among toxic/trace elements, maximum level was recorded for Pb (2.653 mg/kg, FW), followed by Sr (1.604 mg/kg, FW) and Co (1.503mg/kg, FW), whereas other metals were in the order of Cd > Li > Cr without significant difference ($p<0.05$). The dietary reference intakes (DRI) of the elements [47] were compared with the present levels, except Cd and Pb, for which maximum allowed levels (ML) were considered [48]; though Pb, concentration was little high, however, measured levels for other metals were within the safe limits thereby indicating no adverse health effects associated with the consumption of the fruit samples.

3.4. Polyphenol contents. The results showing total phenolics, flavonoids, flavonols and ascorbic acid contents in the fruit of *V. foetens* calculated on fresh weight basis are given in Figure 5. On the whole, total phenolics and flavonols contents were high in acetone extracts, whereas flavonoids and ascorbic acid contents were more in water extracts than corresponding acetone extracts. Phenolics are naturally occurring secondary metabolites in fruits, vegetables, and whole grains [49]. These compounds are derived from phenylalanine and tyrosine and are being used progressively in the food industry because of their inhibitory effect on oxidative degradation of lipids, to improve the quality and nutritional value of food, and their hydroxyl groups bestow scavenging ability [50]. In the present study acetone extract showed more phenolics content compared to water extract with significant difference (p<0.05). Flavonoids possess strong antioxidant capacity [13] and exhibited broad range antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic potential [51]. Comparative evaluation of total flavonoids content (TFC) revealed that aqueous extract exhibited significantly higher amount (113.92 mg Rt/100g, FW) significantly different at (p<0.05) compared to acetone extract, and this level was even higher than reported for the Bitter gourd, Blackberry and Blueberry [52,53]. Total flavonols contents articulated in mg rutin equivalent/100g were higher in acetone extracts (59.90 ± 0.82), whereas water extract contains more value of ascorbic acid (0.563 ± 0.05 mg AA equivalent/100g) was more in water extracts than acetone extracts. These values were significantly different at (p<0.05). Measured values of TPC, TFC and TFlC were in agreement as reported in the edible wild fruits of Meghalaya state India [54]. However, variations in the results might be attributed to the difference in species, genetic variations, harvesting season and geo-climatic factors.

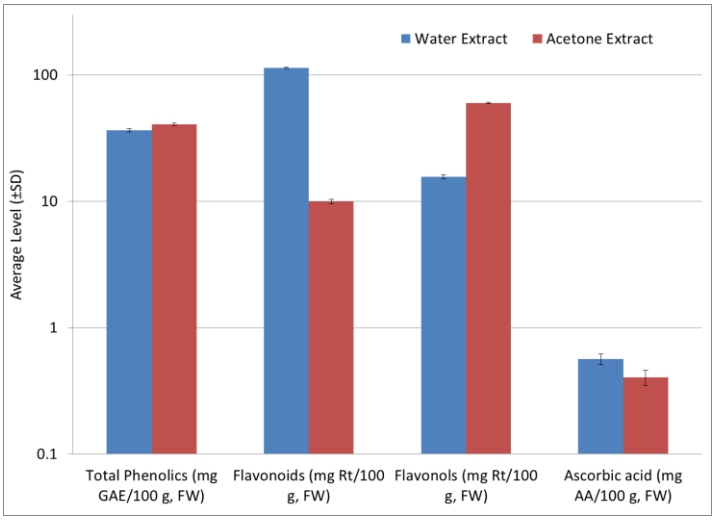

Figure 5. Polyphenol and ascorbic acid contents in *V. foetens* fruit [Each bar shows mean of 3-5 replicates ± SD]

3.5. Free radicals scavenging capacity. Antioxidant capacity of fruits, vegetables, grain, nutraceuticals, dietary supplements and biological fluids and other foodstuffs can be determined by various methods including ferric ion reducing power (FRAP), oxygen radical absorbance capacity (ORAC), 2-diphenyl-1-picrylhydrazyl (DPPH assay), determination of total phenols, 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS assay), hydroxyl radical scavenger activity, superoxide radical-scavenger activity, ferrous ion chelating activity and lipid peroxidation inhibition, which are among the most commonly utilized assays [55-57]. Each method has different experimental technique and principle. Because multiple reaction and mechanisms are usually involved, and no single assay can precisely reflect all antioxidants in a mixed or complex system. Thus, to fully elucidate a full profile of antioxidant capacity, different antioxidant capacity assays may be needed [58]. In the present study, we use six different methods to evaluate free radical scavenging activity in the water and acetone extracts of fruit samples as revealed in Figure 6. These assays include: DPPH, hydroxyl (OH^-) and hydrogen peroxide (H_2O_2) radicals scavenging, ferrous ion chelating (Fe^{2+}), FRAP-assay and phosomolybdenium complex assay (PMA).

Purple colored solution of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), is used to determine the electron donating potency of natural products [13]. Change in DPPH color is related to the concentration and effectiveness of antioxidants [59]. Acetone extract of *V. foetens* fruit exhibited 84.62 ± 0.63 de-colorization of DPPH indicating more scavenging than aqueous extract. A similar observation was reported in *Momordica charantia* Linn. var. *abbreviata* Ser from Taiwan [53]. Hydroxyl radical (OH^-) is one of the most reactive oxygen species intermediary produce in cellular respiration, phagocytic outburst and purine metabolism [60]. OH^- causes damage to almost every molecule of biological system, cell membrane phospholipids and results in carcinogenesis, mutagenesis and cytotoxic effect in the body [61,62]. OH^- radical scavenging capacity of an extract is directly proportional to its antioxidant activity depicted by the low intensity of red color [63]. In the studied samples, water extract exhibited 75.53 ± 0.95 scavenging of OH^- radical which was significantly higher than corresponding acetone extract. Hydrogen peroxide (H_2O_2) is a weak oxidizing agent and present at low concentration in the living organisms, air, water and food [63].

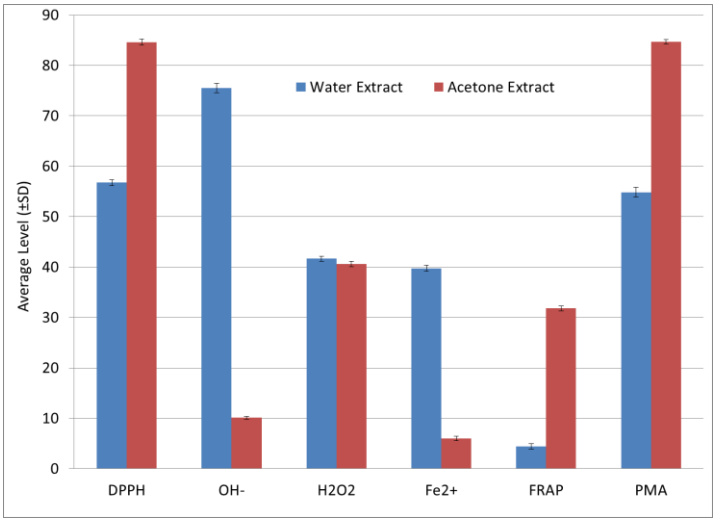

Figure 6. Free radicals scavenging activity of *V. foetens* fruit [Each bar shows mean of 3-5 replicates ± SD]

H_2O_2 is rapidly decomposed and results in the production of hydroxyl radicals (•OH), which instigate lipid peroxidation and causes damage to DNA molecule [64]. In the water extract, H_2O_2 radical scavenging was 41.67 ± 0.49 %, slightly higher than acetone extract. This might be due to the presence of phenolic groups, which could donate electrons to hydrogen peroxide, thereby neutralizing it into water [15]. Iron and copper are essential transition metals in the human body, which activate various enzymes and proteins. These metals are powerful catalysts

of auto-oxidation reactions such as the conversion of H_2O_2 to OH^- in the Fenton reaction and in the decomposition of alkyl peroxides to highly reactive alkoxyl and hydroxyl radicals due to the presence of one or more unpaired electrons [65]. Iron has been concerned in the pathogenesis of Alzhermer's disease, Huntington's disease and Parkinson's disease [66]. Transition metal chelation to form low redox potential complexes is an important antioxidant property [67], and measuring chelation of iron (II) is one method for assessing this property. Aqueous extract of *V. foetens* fruit depicted significant chelation of Fe^{2+} at 39.76 ± 0.55 %, which was relatively higher than previously reported in ethanolic and water extracts of *Zanthoxylum alatum* and *Hyphaene thebaica* [468,69]. An extract with high chelating power reduces the free ferrous ion concentration by forming a stable iron (II) chelate and decreases the level of Fenton reaction which is implicated in many diseases [70].

Usually, the reducing abilities of antioxidants are due to the presence of active compounds which break the free radical chain by donating a hydrogen atom [71]. FRAP assay determines the plummeting potential of an antioxidant reacting with ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a blue-colored ferrous tripyridyltriazine (Fe^{2+}–TPTZ) at low pH [72]. In the present study acetone extract showed more reduction of ferric ions at 31.79 ± 0.47 µM GAE/100g, FW compared to water extract. This value was even higher than reported before in the fruits of *Morus nigra* and *Morus rubra* [73], and in black and blue berries from the Black sea region of Turkey [52]. Total antioxidant capacity (TAC) was determined by phosphomolybdenum complex assay, which is based on the reduction of Mo (VI) to green phosphate/Mo (V) compound. This method is useful for the detection of natural antioxidants such as ascorbic acid, phenolics compounds, tocopherols and carotenoids [34,74], In the edible wild fruit of *V. foetens* acetone extract exhibited comparatively high Mo (VI) reducing ability at 84.67 ± 0.48 µM AAE/100 g, FW than aqueous extract. Present findings revealed that acetone extracts exhibited more inhibition in DPPH, FRAP and PMA assays, whereas measured values of OH^- and H_2O_2 radicals scavenging and Fe^{2+} chelating power were high in the water extracts. Increasing order of antioxidant potential in the water extract was, OH^- > DPPH > PMA > H_2O_2 > Fe^{2+} > FRAP, whereas in the case of acetone extract was DPPH > PMA > H_2O_2 > FRAP > OH^- > Fe^{2+}. All values were significantly different (p<0.05).

3.6. Correlation study. Though a number of phytochemicals including polyphenolic compounds, vitamins, carotenoids, metals contribute to the total antioxidant capacity and free radicals scavenging activity; however which constituents are more responsible are yet to be unknown [58]. Noteworthy correlations between phenolics compounds and antioxidant activity in various fruits have been reported before [12, 75-77]. Nevertheless, antioxidant activity of fruits, vegetables and other foodstuffs is not only because of the phenolics compounds; many other compounds such as polyphenolics phytochemicals, carotenoids, vitamins and metals also contribute significantly in such activities.

In the present study, highly significant correlation coefficients were found in the acetone extracts of *V. foetens* fruit (as shown in Table 1) for flavonoids content with total antioxidant capacity (or PMA assay), Fe^{2+} chelating activity and ferric ion reducing antioxidant power (FRAP assay) (1.000, 1.000 and 0.959, respectively). Likewise, ascorbic acid content (vitamin C) also exhibited strong relationship to PMA assay (r = 0.991), Fe^{2+} chelating activity (r = 0.989) and FRAP assay (r = 0.912). It indicated substantial contributions of flavonoids and ascorbic acid contents towards the antioxidant capacity of the fruits. Similarly, total phenolics exhibited significant relationships with hydrogen peroxide scavenging and ferrous chelating capability. In the case of water extracts, considerable correlations were noted among FRAP and ascorbic acid, total flavonols and flavonoids contents (0.999, 0.985 and 0.962, respectively). Flavonols and ascorbic acid contents also showed strong correlations (at 0.988 and 0.957) with DPPH, respectively. Flavonoids also showed some significantly strong correlations with DPPH scavenging activity. Correlations coefficients were also determined between the metals and antioxidant activity as shown in Table 2. In case of the water extracts, Ca and Sr exhibited highest correlations with H_2O_2 scavenging activity and FRAP value, respectively, followed by Ca-OH^- (r = 0.999), Mn-FRAP (r = 0.996), Mg-PMA (r = 0.995), and Mg-Fe^{2+} (r = 0.992). Among the acetone extracts Cu was significantly correlated with PMA and Fe^{2+} chelating activity (r = 1.000, each), followed by Mn-H_2O_2 (r = 0.995), Co-FRAP (r = 0.993), Ca-DPPH (r = 0.993) and Sr-H_2O_2 (r = 0.972). Some inverse relationships were also observed which showed opposing characteristics of the measured variables. Overall, correlation study revealed significant contributions of essential metals such as Ca, Mg, Mn, Co and Sr towards the antioxidant capability of the fruits.

Table 1. Correlation coefficient matrix between polyphenols and antioxidant capacity in the water extract (below the diagonal) and the acetone extract (above the diagonal) in *V. foetens* fruit.

	TPC	TFC	TFlC	AA	DPPH	OH	H_2O_2	Fe^{2+}	FRAP	PMA
TPC	1	0.608	-0.979**	0.709*	-0.986**	-0.351	0.961**	0.595	0.358	0.607
TFC	0.710*	1	-0.434	0.991**	-0.731*	0.530	0.363	1.000**	0.959**	1.000**
TFlC	0.333	0.900**	1	-0.551	0.932**	0.533	-0.997**	-0.420	-0.161	-0.433
AA	0.462	0.953**	0.990**	1	-0.816*	0.412	0.485	0.989**	0.912**	0.991**
DPPH	0.185	0.823*	0.988**	0.957**	1	0.191	-0.901**	-0.720*	-0.508	-0.730*
OH	-0.709*	-1.000**	-0.901**	-0.953**	-0.824*	1	-0.597	0.544	0.749*	0.531
H_2O_2	-0.734*	-0.999**	-0.885*	-0.941**	-0.803*	0.999**	1	0.348	0.084	0.362
Fe^{2+}	-0.284	-0.877*	-0.999**	-0.981**	-0.995**	0.877*	0.859*	1	0.963**	1.000**
FRAP	0.491	0.962**	0.985**	0.999**	0.947**	-0.962**	-0.952**	-0.975**	1	0.959**
PMA	-0.040	-0.732*	-0.955**	-0.905**	-0.989**	0.733*	0.708*	0.970**	-0.890*	1

*Correlation is significant at p < 0.005 (2-tailed);

**Correlation is significant at p < 0.001 (2-tailed)

TPC-total phenolics content, TFC-total flavonoids content, TFlC-total flavonols content, AA-ascorbic acid content

Table 2. Correlation coefficient matrix between metals and antioxidant capacity in *V. foetens* fruit.

		Ca	Cd	Co	Cr	Cu	Fe	K	Li	Mg	Mn	Na	Pb	Sr	Zn
Water Extract	DPPH	-0.793*	0.967**	0.077	-0.254	0.264	-0.935**	0.807*	-0.254	-1.000**	0.921**	-.825*	1.000**	0.943**	-0.254
	OH	0.999**	-0.937**	-0.637	-0.350	-0.771*	0.968**	-1.000**	-0.350	0.803*	-0.977**	0.349	-0.803*	-0.962**	-0.350
	H_2O_2	1.000**	-0.912**	-0.686	-0.410	-0.811*	0.950**	-0.999**	-0.410	0.763*	-0.961**	0.288	-0.763*	-0.943**	-0.410
	Fe^{2+}	0.850*	-0.988**	-0.176	0.156	-0.359	0.966**	-.0862	0.156	0.992**	-0.955**	0.764*	-0.992**	-0.972**	0.156
	FRAP	-0.941**	0.999**	0.376	0.052	0.545	-0.999**	0.948**	0.052	-0.945**	0.996**	-0.614	0.945**	1.000**	0.052
	PMA	0.714*	-0.930**	0.043	0.367	-0.147	0.887*	-0.731*	0.367	0.995**	-0.868*	0.887*	-0.995**	-0.897*	0.367
Acetone Extract	DPPH	0.993**	-0.961**	-.577	-0.277	-0.721*	0.984**	-0.996**	-0.277	0.846*	-0.991**	0.419	-0.846*	-0.980**	-0.277
	OH	0.125	-0.500	0.655	0.866*	0.500	0.407	-0.148	0.866*	0.721*	-0.371	0.982**	-0.721*	-0.427	0.866*
	H_2O_2	-0.850*	0.988**	0.176	-0.156	0.359	-0.966**	0.862*	-0.156	-0.992**	0.955**	-0.764*	0.992**	0.972**	-0.156
	Fe^{2+}	-0.797*	0.500	0.982**	0.866*	1.000**	-0.588	0.783*	0.866*	-0.240	0.619	0.327	0.240	0.569	0.866*
	FRAP	-0574	0.212	0.993**	0.977**	0.952**	-0.312	0.555	0.977**	0.068	0.349	0.600	-0.068	0.291	0.977**
	PMA	-0.797*	0.500	0.982**	0.866*	1.000**	-0.588	0.783*	0.866*	-0.240	0.619	0.327	0.240	0.569	0.866*

*Correlation is significant at p < 0.05 (2-tailed);

**Correlation is significant at p < 0.01 (2-tailed)

4. CONCLUSIONS

The present study is the first report on proximate nutrients composition, essential/toxic metal levels, preliminary screening of phenolics content and *in vitro* free radicals scavenging capacity and their inter-relationship in the edible wild fruit of *V. foetens*, eaten raw by the inhabitants of the Himalayan region. Edible wild fruits of *V. foetens* revealed significant levels of nutrients, essential metals and phenolics contents; they also possessed strong antioxidant capacity and radicals scavenging action in all tested methods. Significantly, the strong association of the phenolics, ascorbic acid and metal contents with antioxidant capacity showed contribution these components as antioxidants. It revealed that fruits of *V. foetens* are a good source of natural antioxidants and have health benefits for consumers as a potential functional food or value-added ingredient. However, additional studies are desirable to assess the bio-absorption, mechanism of action, and associations between these compounds after consumption.

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6. ACKNOWLEDGEMENTS

Technical and financial support by Quaid-i-Azam University, Islamabad, Pakistan to complete this project is thankfully acknowledged.