

Nutraceutical potential of rose hips of three wild *Rosa* species from Western Himalaya, India

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Abstract

Recently, the use of hips of different rose species has increased due to its nutritional and health-promoting benefits. Rose hips contain a rich amount of ascorbic acid, phenolics, and various other bioactive constituents. Based on the distribution and variation in the morphological characters, rose hips of nine accessions belonging to the three species viz. *Rosa webbiana* Wall. ex Royle, *R. moschata* Herrm., and *R. canina* L. were studied. The rose hips were evaluated for moisture, carbohydrates, total protein, crude fat, total phenolic, crude fibers, water-soluble vitamins, fat-soluble vitamins, and mineral contents. An appreciable amount of the studied biochemical parameters with significant variations was observed in the rose hips originated from different regions. Results demonstrated the high nutraceutical potential of these species and indicated that their rose hips could be promoted as a functional food in the region. Furthermore, the phytonutrient-rich composition of the region's rose hips was revealed when the current study's findings were compared to previous studies. Therefore, they may be considered ingredients in food and dietary supplement formulations, phytopharmaceutical sector and can be quickly brought under cultivation. Furthermore, findings based on the scientific evidence indicate that the information on the pharmacological and toxicological of *R. webbiana* and *R. moschata* is very scanty or lacking; therefore, needing more research interest.

Keywords: ascorbic acid; bioresource; minerals; nutritional composition; *Rosa* species; vitamins; wild food

Introduction

Nowadays, it is projected that approximately 795 million people all over the globe are incapable of securing their basic nutritional requirements to sustain their life. In other words, approximately one over nine persons on earth is undernourished mainly residing in developing countries (FAO, 2015). Wild edibles play a crucial role in bringing down this number as they not only fulfill the energy concerns of the body but also serves as a good source of minerals and vitamins (Datta *et al.*, 2019). The nutritional elements of wild edibles are

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proportionate and sometimes preferable to the cultivated variety (Ebert *et al.*, 2014). As a source of food and medicine, studies are now being concentrated on the wild edibles, especially rose hips due to their rich phytochemical composition (Rosu *et al.*, 2011).

Wild roses belong to the genus *Rosa*, family *Rosaceae* and are widely distributed throughout the Northern Hemisphere (Gu and Robertson, 2003; Wissemann and Ritz, 2005) including North America, Middle East and Asia with Western China harboring maximum of the diversity of *Rosa* species (Phillips *et al.*, 1988). Globally, the genus *Rosa* encompasses about 200 species and 20,000 cultivars (Wissemann and Ritz, 2007). In Indian Himalayan ranges, various *Rosa* species have adapted between an altitude of 500 m and 4700 m above mean sea level and various claims have been made regarding the number of wild roses growing in this region with a maximum of twenty-five species (Rathore and Srivastava, 1992; Tejaswini and Prakash, 2005; Singh *et al.*, 2020a; Singh *et al.*, 2020b).

Among the growing wild rose species of this region, *Rosa webbiana* Wall. ex Royle and *Rosa moschata* Herrm. have a wide distribution and are found growing mostly in dry, rocky areas at higher altitudes, and moist localities respectively (Singh *et al.*, 2020a). Third species *Rosa canina* L., whose hips are widely used as a nutraceutical outside India, is found in Kashmir valley, J&K. *R. webbiana* is a shrub, which belongs to Section *Cinnammomae* with 3-11 leaflets, have pink to red-dish colored flower and red-colored hip with varied shapes, whereas *R. canina* of Section *Caninae* has 3-7 leaflets, red-colored urceolate to obovoid shaped hips and white-colored flowers (Singh *et al.*, 2020a). On the other hand, *R. moschata* of section *Synstylae* have 3-7 leaflets (Singh *et al.*, 2017) and have highly fragrant white-colored flowers and orange to red-colored spheroidal to obovate shaped hips.

Rose hips are the pseudo fruits of roses that develop in a fleshy pericarp and are berry-like structures comprising of an outer hypanthium (fleshy layer), having achenes in-grained inside (Winther *et al.*, 2016). Traditionally, the hips of *R. canina* L. have been consumed for relieving cold, asthma, flu, diabetes, bronchitis, ulcers in different regions of the world (Genc and Ozhatay, 2006; Rexhepi *et al.*, 2013; Karakaya *et al.*, 2019). In India, rose hips are used for the treatment of indigestion, impotency, jaundice, and fever (Kala, 2006; Ballabh and Chaurasia, 2007; Singh, 2012). Whereas the rose hips of *R. moschata* are employed for problems such as leucorrhoea, bleeding, pregnancy termination, gastrointestinal disorders, indigestion (Kumari *et al.*, 2009; Nand and Naithani, 2018). These reports pointed out the potential of these species in improving human health after their proper evaluation and authentication. The rose hips of the wild rose germplasm are abundantly available but neglected and underutilized bioresource of Western Himalayan region, which could be used directly as foods, or used to retrieve valuable bioactive compounds.

Rose hips have got the attention of researchers recently due to the presence of essential phytochemicals in them. They are reported to contain a greater variety of phytochemicals than other fruit species (Olsson *et al.*, 2004). They are also reported to be rich in polyphenolics (Kerasioti *et al.*, 2019), ascorbic acid (Nadpal *et al.*, 2016), minerals elements and vitamins such as A, E, B1, B2, and K (Olsson *et al.*, 2004; Siracusa and Ruberto, 2014; Zivkovic *et al.*, 2015), carotenoids, carbohydrates and fatty acids (Demir *et al.*, 2014). Moreover, the antioxidants, usually coupled to phenolic compounds, are contemplated to have the potential of augmenting the nutritional value and quality of food products (Shahidi and Ambigaipalan, 2015). Rose hips of *R. canina* are utilized in food products, also in many European countries (Gao *et al.*, 2000).

Different extracts of rose hips have been shown to possess antioxidant properties (Ouerghemmi *et al.*, 2016) and have therapeutic actions against inflammation, arthritis, obesity, and ulcers (Gurbuz *et al.*, 2003; Orhan *et al.*, 2007; Nagatomo *et al.*, 2013; Winther *et al.*, 2013; Schwager *et al.*, 2014). The compound 'tiliroside' isolated from *Rosa canina* is known to exhibit anti-diabetic and antiobese activities by increasing the oxidative breakdown of fatty acids in skeletal and liver muscles (Ninomiya *et al.*, 2007; Nagatomo *et al.*, 2013). Several extracts of rose hips are also being sold in the market as health supplements, cosmetics, and as nutritive aid (Patel, 2013). For instance, 'Litozin' (rich in galactolipid GOPO compound), a joint health supplement, has been introduced in the US market by Europharma, Green Bay, Wisconsin, the USA in 2005 (Ohr, 2006). Furthermore, the requirement of less maintenance and wide adaptability of roses across various ecological

habitats makes them potentially profitable crop from food and nutraceutical perspective (Islam, 2013; Singh *et al.*, 2020a).

To the best of our knowledge, nutritional analysis of wild rose hips has not been performed hitherto in India; however, reports are there in the literature regarding the essential oil and fatty acid composition of cultivated roses. The utilization of this important bioresource for rose hip production has a tremendous scope from nutraceuticals and aromatic industrial perspective. Therefore, the present study was conducted in order to explore the availability of hip biomass of economically important *Rosa canina* and the other two unexplored species *viz.* *Rosa moschata* and *Rosa webbiana* collected from different geographical regions of Western Himalaya, India. It also aimed to determine the nutritional composition, mineral content, and vitamin content in the hip (pseudo fruit) samples of these three *Rosa* species for the first time from India. Their consumption by the residents of far-flung areas of the J&K, Ladakh, Uttarakhand and Himachal Pradesh of Western Himalaya was kept in view.

Materials and Methods

Field surveys

To collect first-hand information on the availability of these traditionally useful species of *Rosa*, rigorous field surveys were carried out in the Western Himalayan region from 2016 until 2019. A total of 47 accessions of the selected *Rosa* species *viz.*, *R. moschata* (22), *R. webbiana* (22), and *R. canina* (3) were earmarked (Table S1). Herbarium specimens with all the associated locational and altitudinal data were collected for each accession. The identification of the samples was made using regional floras, regional Herbaria such as Internationally recognized Janaki Ammal Herbarium (RRLH), CSIR-IIIM, Jammu, J&K, and Herbarium of the University of Jammu, J&K (HBJU). Duly identified specimens were submitted to the RRLH. The currently accepted scientific names of the *Rosa* species were confirmed using theplantlist.org. (TPL, 2013). Rose hips of the total of nine accessions of the selected *Rosa* species were collected (Table 1 and Figure S1).

Table 1. Details of the studied wild *Rosa* accessions from J&K and Himachal Pradesh, India

Species	Accession Code	Division, District, State/Union Territory	Locality	Latitude	Longitude	Altitude (m.a.s.l.)	Herbarium No. (RRLH-)
<i>R. webbiana</i> Wall. ex Royle	RW-1	Jammu, Anantnag, J&K	Betab Valley	N 34°03.34'	E 075°21.64'	2440	23549
	RW-2	Kashmir, Bandipore, J&K	Gurez	N 34°28.95'	E 074°57.30'	2378	23565
	RW-3	Jammu, Kishtwar, J&K	Machail	N 33°25.09'	E 076°20.82'	2783	23574
	RW-4	Kashmir, Bandipore, J&K	Bandipore Gurez Road	N 34°28.34'	E 074°37.84'	2195	23514
<i>R. moschata</i> Herrm.	RM-1	Jammu, Kishtwar, J&K	Sohal	N 33°13.91'	E 076°12.01'	1979	23546
	RM-2	Kashmir, Anantnag, J&K	Bijbehara	N 33°53.46'	E 075°10.16'	2119	23534
	RM-3	Jammu, Billawar, J&K	Billawar (Pallan)	N 32°33.14'	E 075°34.14'	792	23531
	RM-4	Shimla, Kinnaur, HP	Rekong	N 31°33.22'	E 078°16.75'	2013	23529
<i>R. canina</i> L.	RC-1	Kashmir, Anantnag, J&K	Betab Valley	N 33°57.48'	E 075°18.04'	2009	23501

Based on the distribution and variation in the morphological characters of *R. webbiana* and *R. moschata* in the study area, four accessions each from these two species were selected for the investigation. Further-more, since the distribution of *R. canina* is restricted to the Kashmir region, only a single accession of *R. canina* was evaluated from this region. The rose hips plucked at different levels of the canopy were collected randomly from three randomly selected plants for each of the selected accession. The air-dried rose hips were crushed to powder form and stored at -20 °C for further analysis. All the following analysis was done in triplicates.

Physicochemical analysis

Analysis of moisture content (%), crude fat (%), carbohydrate (%) and crude fiber (%) of the rose hips were done by employing the AOAC method (AOAC, 2006). The measurement of fresh moisture content was instantly done in the field itself using a portable weighing machine (Brusewitz, 1975).

Determination of mineral elements and vitamins

Dried and powdered rose hips (0.25 g) were put into a Teflon vessel, and HNO₃ was added. The samples were digested in microwave digester. The sample was made up by adding element water to the vessel. After making up the digested sample with element water, 0.2 ml of 10 ppm ISTD internal standard was added. The Tarson tube containing the digested sample and internal standard was run on the ICP-MS machine (Agilent7500) fitted with Fassel type torch, Platinum injector (2.5 mm diameter), Babington high solids nebulizer and Quartz Scott double pass spray chamber.

The measurements were done for 18 minerals which were divided into two groups based on their density i.e., light and heavy metals. The light metals include Aluminum (Al), Barium (Ba), Beryllium (Be), Cadmium (Cd), Calcium (Ca), Magnesium (Mg), and Strontium (Sr). Whereas, Arsenic (As), Chromium (Cr), Cobalt (Co), Copper (Cu), Mercury (Hg), Iron (Fe), Lead (Pb), Nickel (Ni), Zinc (Zn), Selenium (Se), and Manganese (Mn). were placed under light metal group.

Vitamins were determined through high-performance liquid chromatography (HPLC) with a Photo Diode Detector. For fat-soluble vitamins (Vitamins A, D, E, and K), the H-10 column was used while for water-soluble vitamins (Vitamins B1, B2, B6, B12, and C), RP-18 column was used. A mixture of Acetonitrile: Potassium phosphate was used as a mobile phase for water-soluble vitamins while the mobile phase constituted of HPLC Water: Methanol for fat-soluble vitamins.

Determination of total phenolic and protein contents

Sample preparation

500 mg of dried hip sample was crushed to powder form followed with the addition of 1 ml methanol. The mixture was incubated at 40 °C for four hours with constant stirring in the orbital shaker and then centrifuged at 5000 rpm for 5 min. The supernatant was collected in a centrifuge tube. 1 ml methanol was again added to the remaining pellet, and the process of incubation and centrifugation is repeated three times, followed by the collation of the supernatant recovered in the process. The supernatant was dried on the rotavapor, and the dried extracts were dissolved in 1 ml pure methanol and stored at -20 °C till further analysis.

Determination of total phenolic and protein contents

Total phenolic content in the methanolic extract of hips was quantified by employing the method of Ainsworth and Gillespie (2007) with few modifications. Briefly, 20 µl of methanolic extract was mixed in 80 µl of methanol, then 200 µl of 10% (vol/vol) Folin-Ciocalteu's reagent was added to it. After 5 minutes, 800 µl of 700 mM sodium carbonate was added to the mixture which was then incubated for 2 h in the dark. Finally, the absorbance was measured at 765 nm using spectrophotometer. Total phenolic content was calculated as gallic acid equivalent (GAE) per 100g dry weight (DW) using the standard curve of gallic acid ($Y=0.1814x+0.0181$, $R^2=0.9959$). All measurements were taken in triplicates.

The protein content in the rose hips was determined employing Bradford's procedure (Bradford, 1976) with few modifications. Briefly, sample extract of 20 µl was pipetted into the wells of a 96 well plate followed by the addition of 200 µl Bradford reagent and allowed to stand for 2 min. The absorbance of the blue-coloured solution was measured at 595 nm using a spectrophotometer. Total protein content was calculated using the calibration curve generated with standard BSA ranging from 0-2 µg/µl ($Y=0.1350x+0.0053$, $R^2=0.9914$). All the measurements were taken in triplicates, and the results were expressed as g/100gDW.

Statistical analysis

Statistical analysis of the obtained results was done using Minitab 17. Results were presented as mean \pm standard error (SE). Data obtained was statistically analyzed using one-way analysis of variance (ANOVA) to find out the significant mean differences in the studied parameters of the analyzed accessions. Tukey's Post hoc test was used to test differences among means when the F-test was significant ($p \leq 0.05$).

Results

In the current study, the selected three *Rosa* species were found to be widely adapted to varied ecological niches between altitudinal ranges of 792-505 meter above sea level (a.s.l.) (Table S1). Field survey showed that *R. webbiana* is distributed between an altitudinal range of 792-2380 m a.s.l., whereas *R. moschata* is distributed between an altitudinal range of 1605-4504 m a.s.l. It was observed that these two wild species are widely distributed in the Western Himalayan region, and the rose hips of these species are abundantly available. During the entire field survey, *R. canina* was found restricted to the specific locations of Pahalgam region of Anantnag district in Kashmir region (Table S1). Results of the phytochemical evaluation showed significant differences in the values of the chemical constituents both at inter-specific as well as intra-specific levels.

Moisture content

In *R. webbiana*, moisture content (MC) ranged between 32.25-55.09%, with rose hips from Gurez containing the highest, and Betab Valley containing the least MC (Table 2). On the other hand, the average MC in the rose hips of *R. moschata* was in the range of 50.34% (Bijbehara) to 63.68% (Sohal). A significant difference in the MC levels was observed among the studied samples of the three species and even within the samples of a species (Table 2). For instance, in *R. webbiana*, MC content in hips of 'Bandipore Gurez road' was significantly higher than the remaining accessions of *R. webbiana*. Similarly, in *R. moschata*, MC content in hips from 'Sohal' was significantly higher than the remaining accessions (Table 2). Likewise, MC value (48.82%) found in the hips of *R. canina* was significantly higher than the hips of *R. webbiana* from 'Betab Valley' (32.25%) and 'Machail' (39.40%). Whereas the MC content in hips of *R. canina* (48.82%) was significantly lower than the hips of *R. webbiana* from 'Gurez' (55.09%) and *R. moschata* from 'Sohal' (63.68%) and 'Rekong' (55.86%) (Table 2).

Total carbohydrates

The results presented in Table 2 concerning *R. webbiana* showed the highest carbohydrates in the hips from Betab Valley (76.29%), followed by Machail (74.82%), Gurez (74.20%) and 'Bandipore Gurez' (74.13%) localities. In *R. moschata*, percentage value of carbohydrates was highest (80.15) in the hips from 'Billawar'. Carbohydrate content in hips of *R. canina* (81.43%) was significantly higher than the hips of *R. webbiana* (74.13%-76.29%) and *R. moschata* accessions (74.49%-80.15%) (Table 2). In most of the studied rose hip samples, the percentage carbohydrate content values did not vary so much except in *R. webbiana* (Betab Valley), *R. moschata* (Billawar) and *R. canina* (Betab Valley) wherein the differences were statistically significant at $P < 0.05$.

Protein content

Concerning protein content, the current investigation showed protein content in the range of 1.13-3.80 g/100g in the rose hips (Table 2). Among the three species, protein content was highest (3.80 g/100g) in the hips of *R. webbiana* from 'Gurez' followed by *R. moschata* (3.39 g/100g). ANOVA analysis showed significant differences ($P < 0.05$) among the protein concentration values among the hips of *R. webbiana* samples. Similarly, protein content in hips of *R. moschata* from 'Sohal' (3.39 g/100g) was statistically higher than the remaining

R. moschata accessions (1.13 g/100g -1.57 g/100g). It was also statistically higher than all the *R. webbiana* accessions (1.35 g/100g -2.54 g/100g) except for accession from 'Gurez' (3.80 g/100g).

Table 2. Biochemical composition of the studied Rose hips accessions from different locations. One-way ANOVA were carried out separately for each parameter to figure out the differences among different accessions of the studied *Rosa* species. The same letters in a column denote a lack of statistically significant differences between investigated accessions, according to Tukey's post hoc test ($p < 0.05$). Data are reported as mean \pm SE and presented as an average value of three replicates ($n = 3$)

Species	Accession Code	Moisture content (%)	Carbohydrates (%)	Protein content (g/100g)
<i>R. webbiana</i>	RW-1	32.25e \pm 2.08	76.29c \pm 0.42	1.88d \pm 0.24
	RW-2	55.09b \pm 1.46	74.20d \pm 0.10	3.80a \pm 0.03
	RW-3	39.40d \pm 2.38	74.82d \pm 0.37	2.54c \pm 0.15
	RW-4	50.01c \pm 3.69	74.13d \pm 0.41	1.35e \pm 0.14
<i>R. moschata</i>	RM-1	63.68a \pm 1.98	75.09d \pm 0.60	3.39ab \pm 0.15
	RM-2	50.34c \pm 1.52	74.49d \pm 0.38	1.57de \pm 0.07
	RM-3	57.78c \pm 3.37	80.15b \pm 0.44	1.13e \pm 0.08
	RM-4	55.86b \pm 1.00	75.01d \pm 0.40	1.33e \pm 0.21
<i>R. canina</i>	RC-1	48.82c \pm 2.89	81.43a \pm 0.42	3.09b \pm 0.16

RW-1=*R. webbiana* (Betab Valley), RW-2=*R. webbiana* (Gurez), RW-3=*R. webbiana* (Machail), RW-4=*R. webbiana* (Gurez Bandipore Road), RM-1=*R. moschata* (Sohal), RM-2=*R. moschata* (Bijbehara), RM-3 (Billawar, Pallan) RM-4=*R. moschata* (Rekong), RC-1=*R. canina* (Betab Valley).

Crude fat

The crude fat content in the studied rose hips were found to be between 3.84% and 7.19% (Table 3) Our results showed that among the evaluated samples of *R. webbiana*, hips from Betab Valley (7.19%) harboured the highest percentage of crude fat followed by Machail (7.15%) while Bandipore was having the lowest percentage content (4.85%). In *R. moschata*, hips of Bijbehara (6.47%) possessed the highest crude fat percentage, whereas the lowest percentage was obtained for rose hips of Sohal (3.87%). Among all the studied hips samples, hips of *R. canina* contained the lowest percentage (3.84%) of crude fat. The differences in protein content in most of the studied accessions of *Rosa* species were not statistically significant at $P < 0.05$ (Table 3).

Among the three *Rosa* species, percentage crude fat content in hips of *R. webbiana* of 'Betab valley' (7.19%) and 'Machail' (7.15%) were significantly higher than the remaining accessions except for hips of *R. moschata* of 'Bijbehara'

Total phenolic content

Among the three species, total phenolic content was higher in hips of *R. webbiana* from 'Machail' locality than *R. moschata* and *R. canina* accessions. In *R. moschata*, highest total phenolic content was reported in the hips of Rekong locality (320.39 mg GAE/100g). The total phenolic content in *R. canina* was (529.75 mg GAE/100g) and was significantly higher than the remaining accessions except for 'Machail' locality. Likewise, total phenolic content in hips of *R. moschata* from 'Sohal' and 'Rekong' localities were significantly lower than the remaining accessions of the three species (Table 3).

Crude fibers

An appreciable amount of crude fiber was obtained in the studied accessions of the three species. Crude fiber in hips of *R. webbiana* in 'Machail' locality was 46.03% and was significantly higher than the *R. moschata* and *R. canina* (Table 3). In *R. moschata*, the percentage of fiber content ranged from 26.62% (Sohal) to 35.89% (Bijbehara), with all values differed statistically. Crude fiber percentage in *R. canina* (25.72%) was comparable to the *R. webbiana* collected from 'Betab Valley' (26.12%) and *R. moschata* from 'Sohal' (26.62%).

Table 3. Biochemical composition of the studied Rose hips accessions from different locations. One-way ANOVA were carried out separately for each parameter to figure out the differences among different accessions of the studied *Rosa* species. The same letters in a column denote a lack of statistically significant differences between investigated accessions, according to Tukey's post hoc test ($p < 0.05$). Data are reported as mean \pm SE and presented as an average value of three replicates ($n = 3$)

Species	Accession Code	Crude fat (%)	Total phenolic content (mgGAE/100g)	Crude fibres (%)
<i>R. webbiana</i>	RW-1	7.19a \pm 0.12	413.64c \pm 2.93	26.12f \pm 0.30
	RW-2	5.91bc \pm 0.32	119.68h \pm 0.57	40.82b \pm 0.14
	RW-3	7.15a \pm 0.13	586.69a \pm 7.01	46.03a \pm 0.36
	RW-4	4.85de \pm 0.71	303.34e \pm 2.12	21.65g \pm 0.24
<i>R. moschata</i>	RM-1	3.87e \pm 0.39	316.18d \pm 2.78	26.62f \pm 0.36
	RM-2	6.47ab \pm 0.33	170.87g \pm 3.11	35.89c \pm 0.32
	RM-3	4.37de \pm 0.42	184.16f \pm 2.02	32.46d \pm 0.32
	RM-4	5.13cd \pm 0.35	320.39d \pm 4.13	29.06e \pm 0.26
<i>R. canina</i>	RC-1	3.84e \pm 0.15	529.75b \pm 4.59	25.72f \pm 0.47

RW-1=*R. webbiana* (Betab Valley), RW-2=*R. webbiana* (Gurez), RW-3=*R. webbiana* (Machail), RW-4=*R. webbiana* (Gurez Bandipore Road), RM-1=*R. moschata* (Sohal), RM-2=*R. moschata* (Bijbehara), RM-3 (Billawar, Pallan) RM-4=*R. moschata* (Rekong), RC-1=*R. canina* (Betab Valley).

Vitamins

Water-soluble vitamins

In the studied species, vitamin C was determined between 23.44-143.80 mg/100g DW (Table 4). In *R. webbiana*, the hips collected from Machail were found to contain the highest ascorbic acid content (143.08 mg/100g DW) while in contrast lowest ascorbic acid content (21.58 mg/100g DW) was determined for hips of 'Bandipore' locality. Hips of Betab Valley and Machail were significantly different from each other as well from the rest two hip samples of *R. webbiana* (Table 4). Furthermore, the values obtained for this parameter in the rose hips of 'Betab valley' and 'Machail' were statistically different from that of 'Gurez' and 'Bandipore.' About *R. moschata*, the ascorbic acid content ranged between the highest 124 mg/100g (Bijbehara) to the lowest 23.44 (Billawar). All the accessions of *R. moschata* were significantly different ($p < 0.05$) from each other in terms of the amount of ascorbic acid concentration (Table 4). The concentration of vitamin B1 ranged between lowest (0.563 mg/100g) in Betab Valley (*R. webbiana*) to highest (3.373 mg/100g) in Gurez (*R. webbiana*). A significant variation in the concentration was observed at intra as well as inter-specific level. The full concentration water-soluble vitamins were shown in Table 4.

Fat-soluble vitamins

Concerning the fat-soluble vitamins, the highest vitamin A (2.860 mg/100g) and vitamin D (9.333 mg/100g) concentration were determined for *R. webbiana* collected from 'Machail', and lowest (0.46 mg/100g and 2.51 mg/100g) for *R. moschata* procured from 'Billawar' locality respectively (Table 4). In *R. webbiana*, the concentration of vitamin A in 'Machail' and 'Bandipore' localities differed significantly from the other two localities viz., 'Betab valley', and 'Gurez'. In contrast, the hips of 'Bijbehara' showed a significant difference from 'Rekong' (Table 4). Most vitamin E rich hips (9.76 mg/100g) were determined from 'Sohal' (*R. moschata*) locality and least (1.21 mg/100g) from Bandipore (*R. webbiana*) region. Vitamin E content was higher in *R. moschata* in 'Sohal' locality (9.760 mg/100g) than that of *R. webbiana* (1.210 mg/100g-9.60 mg/100g) and *R. canina* (9.293 mg/100g). The concentration of vitamin E in hips of *R. webbiana* from 'Gurez' (9.603 mg/100g) and *R. moschata* from 'Sohal' (9.760 mg/100g) and 'Rekong' (9.293 mg/100g) was almost comparable and was significantly higher than the remaining accessions of the three *Rosa* species. Likewise, the concentration of vitamin K in hips of the three *Rosa* species was almost comparable to each other except for hips of *R. webbiana* from 'Machail' region which has significantly higher concentration of 5.560 mg/100g.

Table 4. Vitamin profile of the studied Rose hips accessions from different locations. One-way ANOVA were carried out separately for each parameter to figure out the differences among different accessions of the studied *Rosa* species. The same letters in a column denote a lack of statistically significant differences between investigated accessions, according to Tukey's post hoc test ($p < 0.05$). Data are reported as mean \pm SE and presented as an average value of three replicates ($n = 3$)

Accession Code	Vit. B1 (Thiamine)	Vit. C (Ascorbic acid)	Vit. A (Retinol)	Vit. D (Calciferol)	Vit. E (Tocopherol)	Vit. K (Phylloquinone)
RW-1	0.563cd \pm 0.12	123.02b \pm 3.00	1.813b \pm 0.26	2.733f \pm 0.20	6.523b \pm 0.21	1.530de \pm 0.23
RW-2	3.373a \pm 0.10	28.85e \pm 1.00	1.503bc \pm 0.20	7.360b \pm 0.28	9.603a \pm 0.27	2.990b \pm 0.20
RW-3	1.113b \pm 0.10	143.08a \pm 3.00	2.860a \pm 0.20	9.333a \pm 0.32	3.100d \pm 0.32	5.560a \pm 0.14
RW-4	0.933bc \pm 0.25	21.58e \pm 3.57	2.420a \pm 0.19	6.900bc \pm 0.29	1.210e \pm 0.33	2.143cd \pm 0.16
RM-1	0.763bcd \pm 0.16	65.73d \pm 3.38	1.173c \pm 0.17	4.580d \pm 0.20	9.760a \pm 0.35	2.550bc \pm 0.19
RM-2	1.153b \pm 0.14	124.00b \pm 5.01	1.073c \pm 0.18	4.703d \pm 0.17	7.170b \pm 0.32	1.113ef \pm 0.28
RM-3	0.800bcd \pm 0.20	23.44e \pm 1.77	0.460d \pm 0.19	2.510f \pm 0.15	3.770d \pm 0.29	0.753f \pm 0.27
RM-4	0.650cd \pm 0.11	96.28c \pm 4.00	1.370bc \pm 0.26	6.280c \pm 0.27	9.293a \pm 0.24	0.543f \pm 0.24
RC-1	0.500d \pm 0.10	117.72b \pm 3.02	1.253bc \pm 0.16	3.563e \pm 0.25	5.120c \pm 0.42	1.753d \pm 0.22

Mineral composition

Among all the 18 minerals in this study, Ca and Mg were present in highest quantity and ranging from 1088.633 mg/kg in Bijbehara (*R. moschata*) to 1779.533 mg/kg in Betab Valley (*R. webbiana*) and 1658.863 mg/kg in Bijbehara (*R. moschata*) to 3191.53 mg/kg in Rekong (*R. moschata*) respectively (Table 5). The Ca and Mg content in the hips of *R. canina* was found to be 1113.563 mg/kg and 1993.203 mg/kg respectively. Ca content in hips of *R. webbiana* from 'Betab Valley' (1779.533 mg/kg) was significantly higher than *R. moschata* (1088.633 mg/kg-1421.900 mg/kg) and *R. canina* accessions (1113.563 mg/kg). Likewise, hips of *R. moschata* from 'Rekong' region were having significantly higher Mg content than the accessions of *R. webbiana* and *R. canina* (Table 5). The reported content of Mn, Cu, Fe and Zn in the present investigation was found in the range of 17.703-53.203 mg/kg, 1.803-11.223 mg/kg, 56.987-744.6 mg/kg and 7.103-12.003 mg/kg respectively.

Table 5. Mineral profile of the studied Rose hips accessions from different locations. One-way ANOVA were carried out separately for each parameter to figure out the differences among different accessions of the studied *Rosa* species. The same letters in a row denote a lack of statistically significant differences between investigated accessions, according to Tukey's post hoc test ($p < 0.05$). Data are reported as mean \pm SE and presented as average value of three replicates ($n = 3$)

Minerals (mg/kg)	<i>R. webbiana</i>				<i>R. moschata</i>				<i>R. canina</i>
	RW-1	RW-2	RW-3	RW-4	RM-1	RM-2	RM-3	RM-4	RC-1
Essential macronutrients									
Calcium (Ca)	1779.533a \pm 1.52	1407.000c \pm 0.45	1334.750d \pm 1.00	1133.470f \pm 0.45	1113.667g \pm 1.03	1088.633h \pm 0.35	1207.000e \pm 1.00	1421.900b \pm 0.47	1113.563g \pm 0.56
Magnesium (Mg)	2854.047b \pm 0.07	2351.203e \pm 0.04	2041.707g \pm 0.06	2530.207c \pm 0.08	2395.800d \pm 0.06	1658.863i \pm 0.08	2189.697f \pm 0.08	3191.053a \pm 0.08	1993.203h \pm 0.06
Essential micronutrients									
Iron (Fe)	81.803f \pm 0.08	117.400d \pm 0.07	132.973c \pm 0.15	741.600a \pm 0.03	60.503h \pm 0.11	78.043g \pm 0.06	87.060e \pm 0.09	236.667b \pm 0.36	56.987i \pm 0.20
Zinc (Zn)	9.603d \pm 0.02	9.403e \pm 0.04	7.903g \pm 0.06	12.003a \pm 0.03	11.400b \pm 0.06	9.803c \pm 0.04	9.203f \pm 0.05	9.803c \pm 0.04	7.103h \pm 0.13
Manganese (Mn)	26.203d \pm 0.09	20.403f \pm 0.07	53.203a \pm 0.04	45.403b \pm 0.04	17.703h \pm 0.08	26.103d \pm 0.08	18.203g \pm 0.07	21.903e \pm 0.04	28.403c \pm 0.05
Copper (Cu)	3.297f \pm 0.03	3.703e \pm 0.06	3.797e \pm 0.05	4.303d \pm 0.09	11.223a \pm 0.05	5.603b \pm 0.09	3.703e \pm 0.03	5.010c \pm 0.10	1.803g \pm 0.05
Nickel (Ni)	2.700a \pm 0.05	0.503d \pm 0.08	0.903d \pm 0.04	1.903b \pm 0.03	0.903c \pm 0.06	0.503d \pm 0.05	0.603d \pm 0.03	0.800c \pm 0.08	0.303e \pm 0.05
Cobalt (Co)	0.033b \pm 0.02	0.043b \pm 0.03	0.050b \pm 0.01	0.300a \pm 0.01	0.023b \pm 0.01	0.043b \pm 0.03	0.012b \pm 0.04	0.067b \pm 0.04	0.011b \pm 0.09

Toxic elements									
Chromium (Cr)	0.203c ± 0.01	0.100cd ± 0.04	0.093cd ± 0.07	1.400b ± 0.09	0.900a ± 0.04	0.070cd ± 0.04	0.073cd ± 0.09	0.103cd ± 0.07	0.043d ± 0.06
Cadmium (Cd)	0.005b ± 0.00	0.008b ± 0.00	0.006b ± 0.04	0.008b ± 0.00	0.008b ± 0.00	0.003b ± 0.00	0.043a ± 0.00	0.004b ± 0.00	0.003b ± 0.01
Mercury (Hg)	0.203d ± 0.04	0.403bc ± 0.09	0.203d ± 0.04	1.500a ± 0.06	0.200d ± 0.05	0.503b ± 0.04	0.303cd ± 0.03	0.503b ± 0.03	0.200d ± 0.03
Aluminum (Al)	42.703d ± 0.28	41.703d ± 0.30	75.800c ± 0.30	488.223a ± 0.54	24.500f ± 0.27	22.203g ± 0.20	33.100e ± 0.26	101.403b ± 0.24	18.600h ± 0.64
Lead (Pb)	0.050c ± 0.09	0.113c ± 0.015	0.090c ± 0.08	0.303b ± 0.03	5.503a ± 0.03	0.073c ± 0.01	0.403b ± 0.03	0.103c ± 0.06	0.050c ± 0.05
Arsenic (As)	0.012b ± 0.00	0.015b ± 0.00	0.023b ± 0.03	0.203a ± 0.02	0.008b ± 0.03	0.009b ± 0.01	0.033b ± 0.01	0.023b ± 0.02	0.065b ± 0.07
Other trace elements									
Strontium (Sr)	36.220b ± 0.04	38.103b ± 0.08	13.203g ± 0.03	11.903h ± 0.08	22.300d ± 0.04	14.303f ± 0.04	35.903c ± 0.04	22.103e ± 0.07	10.903i ± 0.09
Barium (Ba)	8.303f ± 0.05	17.887c ± 0.06	4.213g ± 0.05	10.503 e ± 0.07	11.803d ± 0.04	3.887h ± 0.03	29.403a ± 0.02	22.903b ± 0.07	2.303i ± 0.09
Beryllium (Be)	0.000b ± 0.00	0.000b ± 0.00	0.000b ± 0.00	0.001ab ± 0.00	0.000b ± 0.00	0.000b ± 0.00	0.003a ± 0.00	0.000b ± 0.00	0.000b ± 0.00
Selenium (Se)	0.100b ± 0.04	0.051bc ± 0.00	0.100b ± 0.03	0.203a ± 0.00	0.073bc ± 0.00	0.000c ± 0.03	0.037bc ± 0.02	0.000c ± 0.04	0.000c ± 0.06

PCA analysis

PCA analysis was performed to determine the correlation between biochemical and agro-morphological character of the studied accessions of the three *Rosa* species. As a result of the analysis, it was observed that the rate of variation between the studied accessions of three wild *Rosa* species and their characteristics was 51.85% (Figure 1).

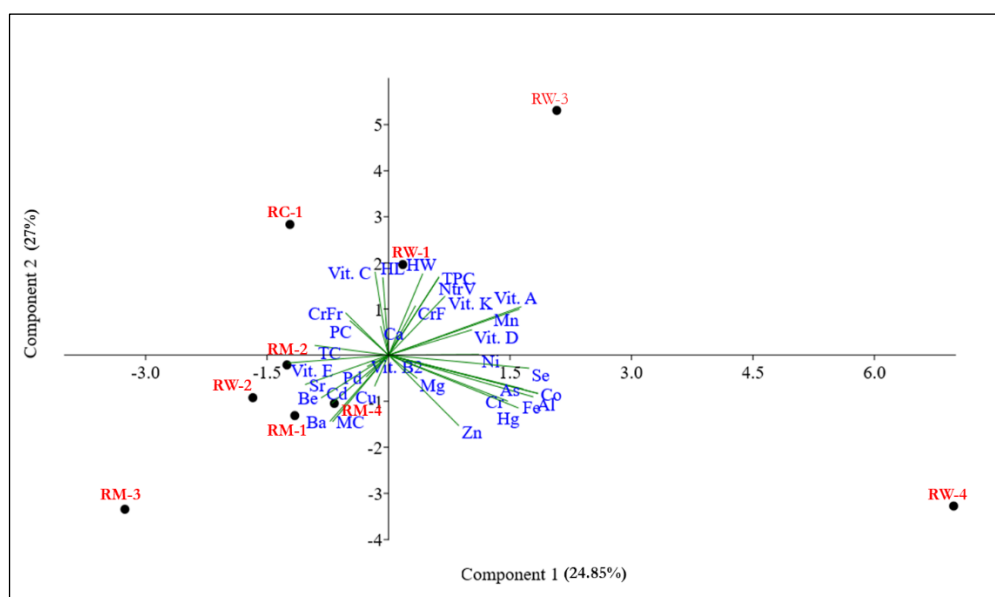


Figure 1. Determining the statistical distribution of wild accessions of the three *Rosa* species according to their biochemical and hip attribute and the change between them by PCA analysis

HL (Hip Length), HW (Hip width), MC (Moisture content), PC (Protein content), TC (Total carbohydrates), CrFr (Crude fat), CrFr (Crude fibre), NtrV (Nutritional value), TPC (Total phenolic content), Vit. B2 (Vitamin B2), Vit. C (Vitamin C), Vit. A (Vitamin A), Vit. D (Vitamin D), Vit. E (Vitamin E), Vit. K (Vitamin K), Ca (Calcium), Mg (Magnesium), Fe (Iron), Zn (Zinc), Cu (Copper), Ni (Nickel), Co (Cobalt), Cr (Chromium), Cd (Cadmium), Hg (Mercury), Al (Aluminum), Pb (Lead), As (Arsenic), Sr (Strontium), Ba (Barium), Be (Beryllium), and Se (Selenium).

PCA shows eight components of which the first two principal components i.e., PC1 and PC2 accounted for 27% and 24.85% of the variance, respectively. The most important variables associated with PC1 were Vit. A, Fe, Mn, Co, Cr, Hg, Pd, As, Se and energy, which were positively correlated with this component. The PC2 was positively correlated HL, HW, NtrV, TPC, and Vit. K, and negatively correlated with MC, Zn and Ba. In the study it was seen that accessions of three *Rosa* species were classified in four different regions. It was determined that RC-1 was located in the first region, RW-1 and RW-3 of *R. webbiana* in the second region, all the accessions of *R. macrophylla* (RM-1 to RM-4) along with RW-2 in the third region and RW-4 in the fourth region (Figure). It was found in the present study that that RC-1 came to the fore in terms of HL, TC, CrFr, Vit. C, PC, and Ca values. The highest HW, TPC, CrF, NtrV, Vit. A, D, K and Mn values were determined in *R. webbiana* accessions RW-1 and RW-3. It was observed that the accessions of *R. moschata* and RW-2 were prominent in terms of Vit. B2, E, Be, Sr, Pd, Cd, Ba and moisture content values, while RW-4 of *R. webbiana* was high in terms of the minerals Co, Mg, Fe, Ni, Cu, Zn, Se, Al, Cr, Hg and As (Figure 1).

In order to highlight possible interdependencies between the nutritional value and the hips attributes of the accessions of three wild *Rosa* species, Pearson correlations were made. The study determined mutual correlations between the studied variables, which can prove dependence between the phytochemicals and the physical attribute of the hips. The study found a strong positive correlation between HL, HW, TPC, Vit. C and NtrV and the values were $r = 0.83$, $r = 0.87$, $r = 1.00$ and $r = 0.66$ respectively indicating that the high nutritional value is of the hips is because of these variables. Furthermore, Vit. C was also found positively correlated with HL, HW, and TPC (Table S2).

Discussion

The average MC in the rose hips of *R. canina* was found to be 48.82%. The average MC in the rose hips of *R. canina* was found to be 48.82% is nearer to the range (49.50-76.53%) reported earlier (Demir and Ozcan, 2001). Furthermore, the MC in the range of 59.65-66.15% was reported for the rose hips belonging to other *Rosa* species by Ercisli (2007), which is comparatively higher than the studied species. The low moisture content in the studied species is considered good from the food safety perspective as low moisture content limits the growth of spoilage microorganisms and enhance their shelf life in storage before processing. Mabellini *et al.* (2011) found 47.7 to 50.8% of moisture content in the hips of *Rosa rubiginosa*, which belongs to the same Section as *R. canina*, i.e., section Caninae. There are no reports of MC in *R. webbiana* and *R. moschata* in the literature that can be compared with the current results.

Carbohydrates are the primary energy source in most countries around the world, therefore their importance in human nutrition cannot be underestimated (Chambers *et al.*, 2019). The hips of the rose species were found to be rich in carbohydrates in the current study. The total percentage of the sugar content of 17.1-27.5% was documented by Najda and Buczkowska (2013) in the rose hips from Poland, which is less than the current results. In the study of Shameh *et al.* (2019) average carbohydrate content in the rose hips of *R. webbiana*, *R. moschata*, and *R. canina* ranged between 203.3-283.2, 104.8-189.2 and 77.8-228.9 mg/g FW respectively. However, in few studies the carbohydrate content reported was found little higher compared to our results (Barros *et al.*, 2011). In horticultural commodities, carbohydrates are the most abundant and widely distributed compounds (Yahia *et al.*, 2019). Carbohydrate content performs a key role in disposing of the sensory quality of ripe fruits (Dugalic *et al.*, 2014).

Protein is an essential component of a well-balanced diet (Wu, 2016). The protein content in the rose hips reported here was higher than reported previously for other species of *Rosa* (Rosu *et al.*, 2011). The protein content reported for *R. canina* in the current study is comparable to the previous report (2.72 g/100g) of Barros *et al.* (2010) whereas a comparatively higher concentration (6.71 to 8.44%) was found by Demir and Ozcan (2001). Furthermore, protein content in the range between 11.50-19.36 mg BAE/100 g was documented in *Rosa laevigata* (Xie *et al.*, 2016).

The crude fat content reported in the present investigation fell well within the ranges documented earlier for different rose species (Nowak, 2005). The reported crude fat contents in the hips of *R. webbiana* and *R. moschata* in the present study were comparatively higher than 1.10% reported recently by Sadia (2020). *R. canina* has a crude fat level of 0.65 g/100 g dry weight (Barros *et al.*, 2010), whereas Ercisli (2007) reported a percentage fat content of 1.52-1.85 for several *Rosa* species, including *R. canina*. The potential health benefit of dietary plant polyphenols as antioxidants has been well established in several studies. Results showed that rose hips in this region have rich potential for total phenolics and can be exploited in the food and pharmaceutical industry owing to their established antioxidant potential (Demir *et al.*, 2014; Fascella *et al.*, 2019). The reported phenolic content in the current investigation is well within the range of 63.76-609.19 mgGAE/100g as reported earlier (Fattahi *et al.*, 2012; Eroglu *et al.*, 2018). Shameh *et al.* (2019) reported phenolic content between 4.69-6.19 mg GAE/g FW in *R. webbiana*. The wide variation found in this study substantiated the dependence of polyphenols on environmental conditions as well as a specific type, as stated in earlier studies (Macheix *et al.*, 2005; Siracusa and Ruberto, 2014; Deng *et al.*, 2015).

An appreciable amount of crude fibre was obtained in the studied accessions of the three wild *Rosa* species for the first time from India. The importance of fibers for digestive health is much appreciated (Barber *et al.*, 2020). The percentage of crude fibre in *R. canina* reported in this study (25.72%) was substantially greater than the previously reported range of 3.27-4.03 percent (Demir and Ozcan, 2001). The crude fiber content found in this study is comparable to that of high-fiber cereals such as rye (17.9%), maize (18.7%), wheat (21.3%), and barley (42.2%) (Rodehutschord *et al.*, 2016). The studied hips of the *Rosa* species serve as a possible candidate in the formulation of fiber rich food products, considering the rich fiber content determined in this study.

The current study of ascorbic acid in rose hips is significant because ascorbic acid is an important dietary antioxidant that is found primarily in fruits and vegetables and performs variety of functions in the body (Padayatty *et al.*, 2003; Santos and Silva, 2008). Vitamin C is a water-soluble vitamin playing a pivotal role in scavenging the free radicals, which are detrimental to the body, especially the plasma membrane (Arrigoni *et al.*, 2002; Wu *et al.*, 2017). Vitamin C reported in the present study was well within the earlier reported range of 109.67-215.14 mg/100g FW (Najda and Buczkowska, 2013). The reported vitamin C content in *R. webbiana* is lower than the earlier reported range of 60.58-75.32 mg/g FW in North West Iran (Shameh *et al.*, 2019). A considerable amount of ascorbic acid (117.72 mg/100g) was found in *R. canina* which was higher than the earlier report of Barros *et al.* (2010) (68.04mg/100g DW). They were lower than those reported by Adamczak *et al.* (2012) (510 mg/100g DW) and Fascella *et al.* (2019) (513.9/100g DW). Earlier research reports have shown the presence of ascorbic acid in the range of 106-2712 mg/100 g from different rose species growing in different climatic regions of Turkey (Demir and Ozcan, 2001; Ercisli, 2007; Yoruk *et al.*, 2008; Gunes and Sen, 2001). Hips of *R. canina* are known to have high antioxidant properties owing to the high content of vitamin C and flavonoids (Tumbas *et al.*, 2012). For men and women, the Recommended Dietary Allowances (RDAs) are 90 mg and 75 mg, respectively, and the Tolerable Upper Intake Levels is 2000 mg per day for both (Fan *et al.*, 2014). The role of vitamin D in influencing bone mineralization by promoting the intestinal absorption of phosphorus and calcium is well appreciated (Morris *et al.*, 2012; Schwalfenberg and Genus, 2015). There were no published studies on vitamin D concentrations in rose hips that could be compared to the current results.

Vitamin E is known to possess antioxidant property and is believed to protect the oxidative destruction of cell membranes (Rizvi *et al.*, 2014). Regarding vitamin E, just a single report was traced in the literature in which α , β , and γ -tocopherols were found to occur in the concentration of 0.19-7.05 mg/100g DW (Barros *et al.*, 2010), which was comparable to our results. Vitamin K performs a vital role in the formation of blood clotting factors (Vermeer and Schurgers, 2000). There was no previous research on vitamin K concentrations in rose hips that could be compared to the current results. The recommended daily allowance (RDA) of calcium for adults is 1000 mg (Khan *et al.*, 2011). Therefore, the studied rose hips are capable of contributing appreciably to the RDA for calcium. These mineral elements are very beneficial from health

perspective as these acts as cofactors for many important metabolic enzymes inside the body. The living organism requires minerals for performing the necessary function of life, which is evident from various reports in the literature (Berdanier *et al.*, 2013). The previous results of Kazaz *et al.* (2009) regarding the concentration of Ca (6301.0 mg/kg), Mg (1652.0 mg/kg), Mn (32.0 mg/kg), Fe (27.0 mg/kg), Cu (4.0 mg/kg), and Zinc (10.0 mg/kg) fell within the ranges of our results. The RDA for Mn, Cu, Fe, and Zn for Indians is 4 mg/day, 1.7 mg/day, 17-35 mg/day, and 10-12 mg/day (FSSAI, 2020). The reported content of these mineral elements in the present study therefore can contribute substantially to the RDA level. The toxic mineral elements such as As (0.008- 0.203mg/kg), Ni (0.303- 2.7 mg/kg), Cr 0.043-1.400 mg/kg and Pb (0.050 mg/kg-5.503 mg/kg) were found in trace amounts in the present study, which gives an advantage to these hips of being used in the food industry. The concentration of Cr (<0.018 mg/kg) and Pb (3.00-3.34-15.3 mg/kg) reported by Zeiner *et al.* (2018) in rose hips were comparable to the results of the current stud whereas Ni concentration (10.6-11.3-23.5mg/kg) was lower than the results of current study.

Conclusions

This study is performed for the first time from India concerning the three *Rosa* species. These wild *Rosa* accessions are explored from the high-altitude regions of the study area, and are important in terms of conserving genetic diversity, and providing materials for future breeding studies and developing *Rosa* genetic resources. Given the high carbohydrate content, the hips of the studied *Rosa* species can be consumed for satisfying the hunger intent as well as provide an edge from the industrial aspect. Comparing the current study results with the already existing reports elsewhere also showcased the phytonutrient-rich nature of the region's rose hips and may be considered as an ingredient in the food and dietary supplement formulations for the local people as well as in the phytopharmaceutical sector. Furthermore, the low content of the toxic mineral elements such as Pb, As, Cd, Ni, Cr, in the majority of the rose hips of different localities are also advocating towards their importance of being used as food. The phytochemical variation observed in rose hips from different regions could prove helpful in the identification of elite accessions or species for genetic improvement programs. The correlations found between physical-chemical parameters of examined accessions can be exploited by the rose breeders, consumers for developing elite genotypes in future. Extensive field surveys and in-depth biochemical characterization of hips of rose species in this region are therefore warranted, which could lead to the identification of elite genotypes or species with strong potential from a future food security perspective.

Authors' Contributions

Designed the study: SG; Data Collection: KS, JFL, SB; Analysed the data: KS, DS, SG; Wrote the manuscript: KS; Revised the manuscript: SG, DS and YP. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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