Indian Journal of Natural Products and Resources
Vol. 13(3), September 2022, pp. 339-345
DOI: 10.56042/ijnpr.v13i3.52195

Antioxidant activities of dried wild rosehips (*Rosa moschata*) of Kullu Valley, Northwestern Indian Himalaya

Sarla Shashni* and Sheetal Sharma

G. B. Pant National Institute of Himalayan Environment, Himachal Regional Center, Mohal-Kullu 175126, Himachal Pradesh, India

Received 07 July 2021; revised received 09 June 2022; accepted 07 July 2022

Wild rose species *Rosa moschata* Herrm. syn *R. brunonii* Lindl. belongs to the Rosaceae family. It has numerous medicinal properties and hence is used to make tea, oil, jam, juice, etc. around the world. The species is found abundantly in Kullu Valley of Himachal Pradesh, Northwestern Indian Himalaya. It is considered as weed and is left unattended in the region. The study aimed to assess antioxidant property of the rosehips to establish an enterprise through its value-added product development, especially tea. Results showed rosehip flesh extract yields as 37.92% in water and 39.06% in methanol respectively. Study showed total phenolics content of 660±1.52 mg GAE/g in water and 675±2 mg GAE/g in methanol extract and total flavonoid content of 498±0.50 mg Rutin/g in water and 557.33±0.57 mg Rutin/g in methanol extract. The antioxidant activities were determined by DPPH IC_{50} values as 2.72±0.01 AAE µg/mL in water and 1.48±0.09 AAE µg/mL in methanol; ABTS assay as 14.10±2.51 GAE µg/mL in water and 22.68±1.83 GAE µg/mL in methanol; FRAP assay as 32±3.14 µM ascorbic acid equivalent/100 g DW. The study showed that the rosehip species has a high antioxidant property which supports its use as beverages for various health benefits.

Keywords: Antioxidant, Beverages, Health benefits, Kullu Valley, *Rosa moschata* Herrm., Rosehip.

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 131/00, A61P 39/00

Introduction

In recent times, there is a growing global interest in herbal products having antioxidant properties. Antioxidants are defined as a defence system used by the body during various physiological processes against the damage of reactive oxygen system[1]. These defensive effects might be due to the characteristic mechanism of secondary metabolites like flavonoids, phenolics, terpenoids and alkaloids[2]. Natural antioxidants of plants, fruits, vegetables, beverages, etc. are considered safe and are major research criteria to determine its inhibition rate against free radicals generated in the human body[3]. Therefore, natural antioxidants are used by consumers worldwide for various health benefits and lowering consequences caused by synthetic antioxidants[4,5]. These natural antioxidants are also known to prevent the risk of diseases like coronary heart disease, cancer, diabetes, and inflammatory diseases[6]. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), and propyl-gallate (PG) are commercially available and used in the food industry[4]. These are quite unsafe due to their toxicity and carcinogenicity as compared to the natural antioxidants like phenolics and flavonoids, which are safe and bioactive[7]. These are also referred as endogenous sources of antioxidants present in the body and exogenous as food sources[1]. Endogenous system of the human body generates some free radicals such as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) because of exposure to different pathological and physiological stress[8]. Free radicals are molecules capable of independent existence which contain an unpaired electron in an atomic orbital and their adverse effects are also documented[9]. Due to inappropriate diet and lack of awareness in consumers, free radicals are badly altering human health and even lead to diseases like cancer and coronary heart diseases[8].

A number of medicinal plants and wild edibles are used by humans as spice, beverage and to cure various ailments and diseases from ancient times[7]. Of these, herbal teas which are infusion of dried plant parts steeped in boiling water are the second most consumed beverage worldwide after water for various

*Correspondent author
Email: sarla.shashni@yahoo.co.in

therapeutic properties[10]. Herbal tea is made of either one ingredient or blend of ingredients consumed for specific purpose like relaxation, rejuvenation and get relief from some specific condition[11]. Large number of companies producing and marketing herbal tea worldwide shows it demand[12]. Among various species of rosehips, several rosehip species are also used in herbal tea having high vitamin C content and tonic for liver, kidney, and blood and these teas are used as a remedy for cough, cold and fatigue[13]. *Rosa moschata* Herrm. syn *R. brunonii* Lindl. is native to Afghanistan, Pakistan, Nepal, Si-Chuan, Europe, South Africa, and South Andes[14]. There are more than 120 species of *Rosa* documented worldwide and around 25 species are reported in India only[15,16]. Of the species reported from India, 5 species of wild roses are grown in the state of Himachal Pradesh in which species of *R. moschata* is found abundantly in the Kullu district[16]. The suitable habitat for the plant species is degraded land, near water streams and forests. Various plant parts such as flower, roots and fruit-bearing seeds have wide applications in the therapeutics with antimicrobial, diuretic, dermatitis, antispasmodic, eczema and anti-ageing properties[17]. It is also reported that analysis using standard procedures can differ in the composition of compounds depending on the altitudinal variation and climatic conditions[18].

The species is abundant in higher altitudes of the Garsa valley with an altitudinal range of 1306 to 1828 m above msl with latitude and longitude range from 31’50.899 N to 077’13.987 E and 31’52.539 N to 077’13.813 E. Traditional use of the species and potential to develop rosehip-based enterprise has also been documented[19]. The current study is to assess the natural antioxidant activities in the dried wild rosehips pods (*R. moschata*) of the northwestern Himalayan district of Kullu, Himachal Pradesh. Antioxidant property of the rosehips will be helpful to harness its nutritional as well as economical aspects, especially by the women in the region for their overall social and economic upliftment.

Materials and Methods

Plant collection and identification

The plant samples of *R. moschata* carrying leaves and flowering parts were collected from the field (Garsa at an altitude of 1530 m amsl during May 2019) and pressed in herbarium sheet. The specimen was further identified by Dr S. S. Samant, Scientist G in the Institute using flora of Himachal Pradesh[20], flora of Kullu district[21], and literature[22,23].

Plant materials and chemicals

The matured *R. moschata* fruits or rosehips were collected from Garsa Valley of Kullu district, Himachal Pradesh during November 2019. The samples were stored and transported in zip-lock polythene bags to the Institute laboratory for further processing. Samples were cleaned thoroughly to remove the overlying dust, twigs, leaves, and contaminant particles. The samples were then sun-dried for the first day and then allowed to shade dry for next 20-25 days. The Rosehip pod part was separated or cut through seed shredder machine and sieved for removal of hairy part and seed. The pod covering part was again grounded (which was used for tea preparation) in mortar pestle to small pieces for further analysis.

Chemicals for analysis like 2,2-diphenyl-2-picryl hydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), aluminum chloride, ferric chloride, ferrous sulphate septahydrate, gallic acid, ascorbic acid, rutin, Folin-Ciocalteu’s phenol reagent, ferrous sulphate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Sodium nitrite were purchased from Hi-Media and CDH representatives.

Preparation of extract

Extract preparation was performed according to Chandra *et al*. with slight modifications[24]. Shade dried, cleaned, and grounded Rosehip pod cover (20 g) was boiled for 30 minutes in 100 mL of water and 80% methanol to obtain the extract. The solvent extract was then evaporated under reduced pressure at 40ºC using a rotary evaporator to obtain semi-solid materials. The semi-solid material is then processed using lyophilizer. The semi-greasy extract is obtained after lyophilization which yields 37.92 and 39.06% in water and methanol respectively. The extract was stored in 4ºC for further analysis.

Total phenolic content

The total phenolic (TPC) content was determined using the Folin-Ciocalteu method[7]. Precisely 200 µL sample (10 mg/mL) was made up to 3 mL using distilled water, to which 500 µL of Folin-Ciocalteu’s reagent was added. The mixture was sonicated thoroughly for 3 minutes. Then, 2 mL of 20% (w/v)

sodium carbonate was added and incubated for 1 h in dark. The absorbance was recorded at 765 nm using UV-Spectrophotometer (Ultrospec 2100 Pro, Healthcare Biosciences AB, Uppsala, Sweden). The total phenolic content was calculated as gallic acid equivalent (GAE) by the following equation[25]:

$$TPC = (C \times V) \div M$$

where TPC is the total phenolic content in GAE mg/g, C is the concentration of gallic acid obtained from the standard calibration curve, V is the volume of the extract solution in mL and M is the weight of the extract in g.

Total flavonoid content

The total Flavonoid (TFC) content was analyzed by the aluminium colourimetric method[26] with slight modifications. Exactly 50 µL (10 mg/mL ethanol) was made up to 1 mL with 80% methanol and 4 mL of distilled water with 300 µL of 5% $NaNO_2$ were mixed in the solution. The mixture was allowed to stand for 5 minutes and 300 µL of 10% $AlCl_3$ was mixed in the solution. After 6 minutes, 2 mL of sodium hydroxide solution was added and the final volume was made up to 10 mL with distilled water. The solution was mixed thoroughly by sonication and the absorbance was recorded after 15 minutes at 510 nm using UV-Spectrophotometer. The total flavonoid content was calculated using the equation[25]:

$$TFC = (C \times V) \div M$$

where TFC is the total flavonoid content in rutin equivalent per g dry weight, C is the concentration of rutin obtained from the standard calibration curve, V is the volume of the extract solution in mL and M is the weight of the extract in g.

Antioxidant properties

2,2-Diphenyl-2-picryl hydrazyl radical scavenging assay

DPPH (2,2-Diphenyl-2-picrylhydrazyl) radical scavenging activity was performed according to the free radical method[27] with slight modifications[28]. In brief, 1 mg/mL stock solution was diluted to a series (25-200 µg/mL) with 70% (v/v) methanol. Then, 2.8 mL 0.06 mM DPPH was mixed and the absorbance at 517 nm against methanol as a blank was recorded using UV-Spectrophotometer after incubation at 37ºC for 30 minutes. The DDPH radical scavenging activity as percentage inhibition was calculated by the equation:

$$\% \text{ inhibition} = [(\text{Abs control} - \text{Abs sample}) \div \text{Abs control}] \times 100$$

The standard calibration curve was plotted against different concentration and DPPH scavenging. The concentration of the sample necessary to decrease the DPPH concentration by 50% was obtained by interpolation from linear regression analysis and denoted as IC_{50} value (µg/mL).

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid assay

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay were performed with some modifications to the decolouration method[29]. About 7 mM ABTS aqueous solution was mixed with 2.4 mM potassium persulphate (PPS) in the ratio of 1:1 (v/v) and allowed to stand for 12-16 h for the formation of free radical. The mix was diluted in ethanol (1.89v/v) to achieve absorbance 0.700±0.02 at 734 nm for further use. The mixture is required to dilute in ethanol (1.89) to achieve the absorbance of 0.700±0.02 at 734 nm. Different concentration (1.56-25 µg/mL) of the sample with ABTS solution was made and the absorbance was recorded using UV-Spectrophotometer at 734 nm after incubation for 15 minutes. The antioxidant activity of the tested sample was calculated by determining the decrease in absorbance at a different concentration by using equation:

$$\% \text{ inhibition} = [(\text{Abs control} - \text{Abs sample}) \div \text{Abs control}] \times 100$$

The standard curve of gallic acid was made between different concentrations and percentage inhibitions to achieve equation for calculating of IC_{50} value.

Ferric-reducing antioxidant power assay

FRAP (Ferric-reducing antioxidant power) assay was performed as per Benzie and Szeto, 1999 with slight modifications[30]. The stock solution was prepared by mixing 300 mM acetate buffer with pH 3.6, 10 mM TPTZ in 40 mM HCL, and 20 mM $FeCl_3.6H_2O$ solution. The working solution included 25 mL of acetate buffer, 2.5 mL of TPTZ, and 2.5 mL of $FeCl_3.6H_2O$. Different dilutions were made using sample, distilled water and FRAP reagents 0-100 µL. A blank solution was prepared by mixing of 2 mL FRAP reagent with 1 mL H_2O. All the concentrations for recording absorbance were allowed to stand for 30 minutes in the dark and after that optical density was recorded at 593 nm using UV- Spectrophotometer.

Statistical analysis

The experimental design includes triplicate samples and performed twice to check the

reproducibility of experiments. The values were given as mean of triplicate ± standard deviation (SD). All the statistical analysis was carried out using SPSS version 16.0. The confidence level at P <0.05 was considered as statistically significant. Graphs were plotted using Microsoft Office Excel 2007.

Results

Total phenolics and flavonoid contents

The total phenolic content was determined using gallic acid as the reference compound. The total phenol was found to be 660±1.52 mg GAE/g in water extract and 675±2 mg GAE/g methanol extract using equation $y = 0.001x - 0.027$ (R^2=0.999) established from gallic acid standard calibration curve where y is absorbance at 765 nm and x is total phenolic content in Rosehip pod extract (Fig. 1). Total Flavonoid content was determined as 498±0.50 and 557.33±0.57 mg Rutin/g in water and methanol extract, respectively using equation $y = 0.001x + 0.007$ (R^2=0.995) established from rutin standard calibration curve where y is absorbance at 510 nm and x is total flavonoid content in Rosehip pod extract (Fig. 2).

DPPH radical scavenging assay

DPPH radical scavenging assay is mostly used assay for determining the antioxidant potential of herbal extracts and phytochemicals. The amount of sample decreases the initial DPPH concentration by 50% is denoted by IC_{50} value which is observed 2.72±0.01 AAE µg/mL in water extract ($y = 0.016x + 6.462$; R^2= 0.995) and 1.48±0.09 AAE µg/mL in methanol extract ($y = 0.031x + 3.982$; R^2=0.994). Ascorbic acid was taken as reference which shows IC_{50} value 3.9±3.2 µg/mL ($y = 0.057x + 6.153$; R^2=0.983) (Fig. 3).

ABTS and FRAP assay

The ABTS assay is also used worldwide for determining the radical scavenging activity of plant extract. The ABTS radical scavenging activity is determined using gallic acid as the reference compound. The IC_{50} value was calculated by plotting inhibition percentages graph against different concentrations and achieving equation ($y = 2.744x - 2.033$; R^2=0.996) (Fig. 4). The IC_{50} value for gallic acid was observed to be 18.96±0.62 µg/mL and extract showed significant results in terms of IC_{50}

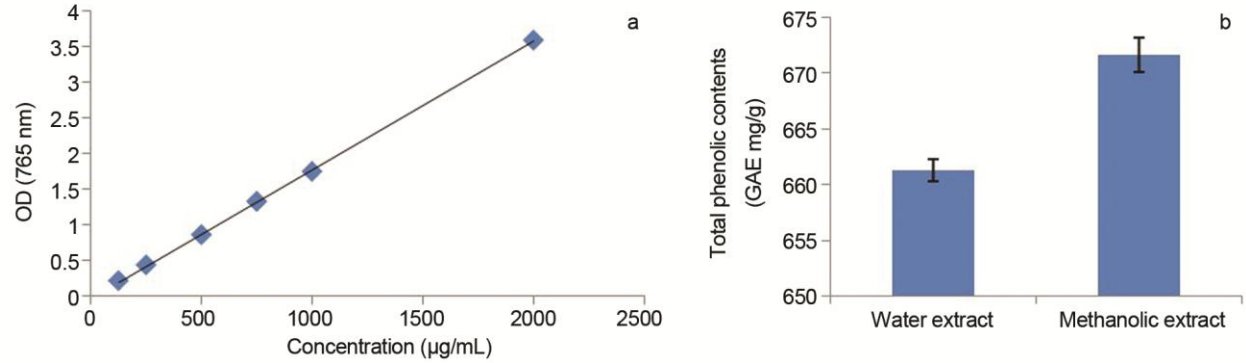

Fig. 1 — a) Standard curve of absorbance against Gallic acid concentrations (µg/mL) y = 0.001x-0.027; R^2 = 0.999; b) Total phenolics content Gallic acid equivalent mg/g DW in Water and Methanolic rosehip extract. The values were expressed as mean±standard deviation.

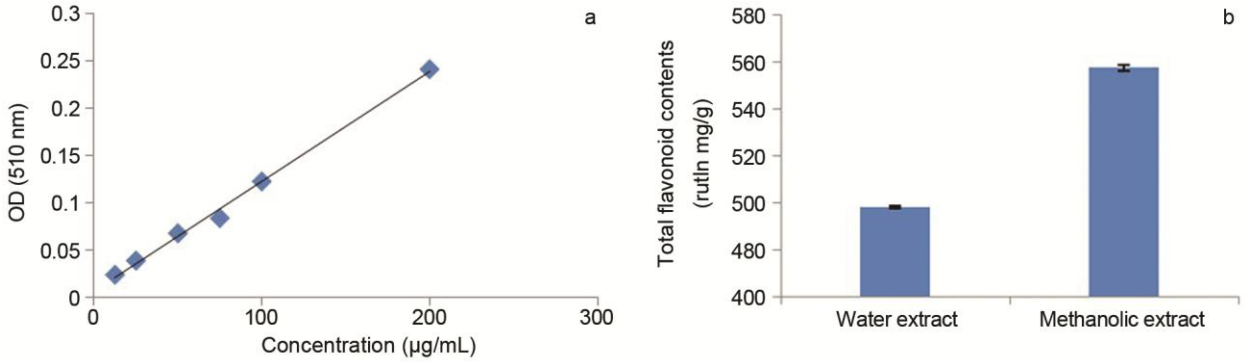

Fig. 2 — a) Standard curve of absorbance against Rutin concentrations (µg/mL) y = 0.001x+0.007; R^2 = 0.995; b**)** Total flavonoid content Rutin Equivalent mg/g DW in Water and Methanolic rosehip extract. The values were expressed as mean±standard deviation.

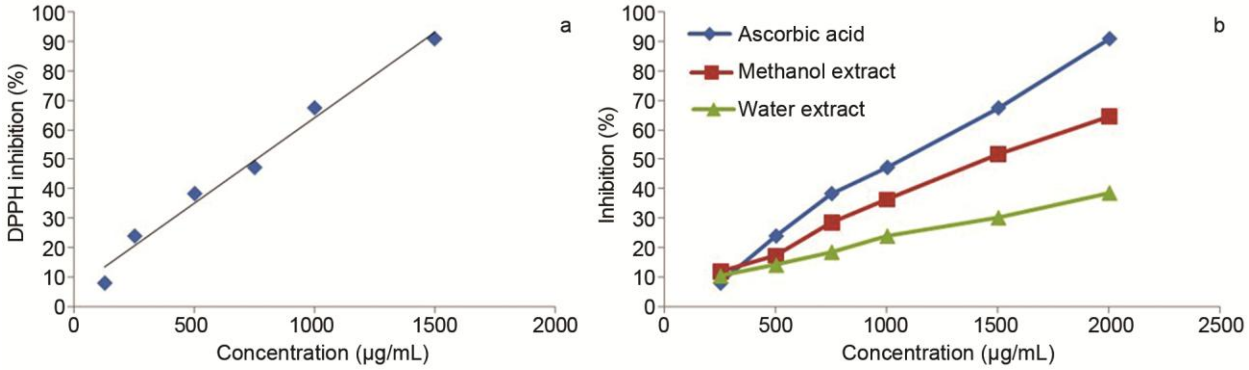

Fig. 3 — a) Ascorbic acid standard calibration curvey=0.057x+6.153, R^2=0.983; b**)** Free radical scavenging DPPH activity in Methanol and Water extract of Rosehip flesh in comparison to Ascorbic acid as a positive control. The values were expressed as mean±standard deviation.

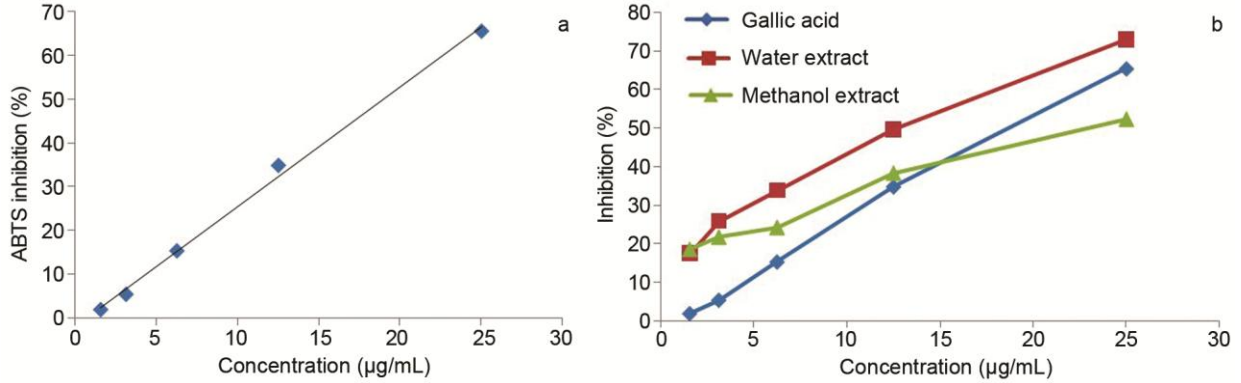

Fig. 4 — a) Gallic acid standard calibration curve y=2.744x-2.033, R^2=0.996; b) Free radical scavenging ABTS activity in Methanol and Water extract of Rosehip flesh GAE µg/mL. The values were expressed as mean±standard deviation.

value, 14.10±2.51 and 22.68±1.83 GAE µg/mL in water and methanol extract respectively. Antioxidant activity determined through FRAP assay was 32±3.14 µM ascorbic acid equivalent/100 g DW, which was calculated by plotting graph between different concentration and absorbance achieving the equation $y = 0.002x + 0.066$; R^2=0.986 (Fig. 5).

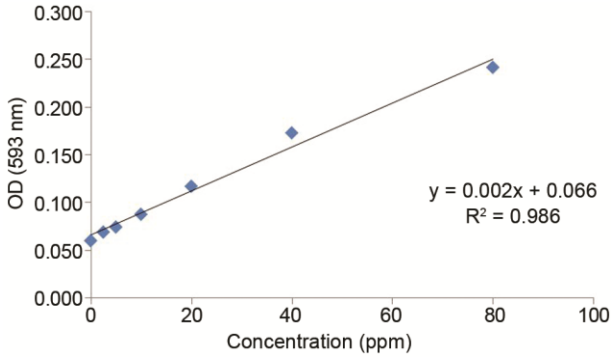

Fig. 5 — Standard calibration curve of Ascorbic acid used to calculate FRAP value as Ascorbic acid equivalent (µM). The values were expressed as mean±standard deviation.

Discussion

Rosehip pod extract yielded 37.92% in water and 39.06% in methanol. TPC and TFC shows good results in the case of methanolic extract as compared to the water extract which is similar to another study[31]. The reason for it could be due to solubility difference and the free 3-OH group[7]. Phenolics content of *R. canina* in water extract was reported as 326 to 575 mg GAE/100g DW[32] and 818 mg GAE/100g DW[33]. While in methanolic extract, this ranged 180 to 225 mg GAE/100g[31], 102.9 mg GAE/100g[32] and 149.35 mg GAE/100g[33], respectively. In *R. rugosa*, it is reported as 215.14 mg GAE/100g DW, 121.38 mg GAE/100g DW[34]. The reason for it could be due to solubility difference and the free 3-OH group[7]. Phenolics content of *R. canina* in water extract was reported as 326 to 575 mg GAE/100g DW[32]. Phenolics content of *R. canina* in water extract was 818 mg GAE/100g DW[33]. While in methanolic extract, this ranged 180 to 225 mg GAE/100g[31]. Another study reported the range between 102.9 mg GAE/100g[32] and 149.35 mg

Table 1 — Total phenolic content in different Rose species

Solvent	*Rosa moschata (Kullu Valley)* (mg GAE/g DW)	*Rosa canina*[1] (mg GAE/ 100 g DW)[32]	*R. spinosissima*[2] (mg GAE/ 100 g DW)[31]	*R. rugosa*[3] (mg GAE/ 100 g DW)[34]	*R. gallica*[4] (mg GAE/ 100 g DW)[35]
Water	660±1.52	326-575	121.38	215.14	3151
Methanol	675±2	102	-	-	-

GAE/100g[33], respectively. In *R. Rugosa* has reported 215.14 mg GAE/100g DW, 121.38 mg GAE/100g DW[34] and 150.8 to 299.2 mg GAE/100g DW in *Rosa spinosissima*[31]. Highest TPC has been reported as 3151 mg GAE/100g DW in *R. gallica*[35] (Table 1).

In the case of flavonoid content, it is reported as 169.49±1.28 mg Rutin E/100g FW in water extract and 194.82±3.46 mg Rutin E/100g FW in acetone extract of *R. moschata* (syn *R. brunonii*) from Pakistan Himalaya[5]. Compared to which, the present result gave 498±0.5 mg Rutin E/100g DW in water extract and 557.33±0.57 mg Rutin E/100 g in methanol extract.

Numerous studies have been reported on the different bioactivities of the plant extract[32-34]. Ascorbic acid being one of the essential nutrients in the human diet is taken as a reference in the free radicals scavenging assays[36]. For the determination of antioxidants, ascorbic acid and gallic acid was taken as reference and IC_{50} value of reference compound and rosehip extract was compared. The IC_{50} value for DDPH per cent inhibition was found 2.72±0.01 µg/mL in water and 1.48±0.09 µg/mL in methanol against 3.9±3.2 µg/mL in ascorbic acid. In the case of ABTS assay, the IC_{50} was recorded as 14.10±2.51 µg/mL in water and 22.68±1.83 GAE µg/mL in methanol extract against 18.96±0.62 µg/mL of gallic acid. FRAP activity for the rosehip extract was found 32±3.14 µM ascorbic acid equivalent /100g DW. Some reports[32,34,35] also suggested FRAP assay an appropriate evaluation method for total antioxidants in plants which are consumed by humans because thiols are the only compounds with which FRAP does not react[37]. It is reported that lower IC_{50} value of the extract means higher antioxidant activity in the sample[38]. Flavonoids in tea have high antioxidant and radical scavenging activities which is also rich in rosehip tea, Rosehips tea has high concentration of flavonoids and high radical scavenging activities; therefore, it can be consumed as a natural source of antioxidants. These bioactive compounds help in neutralizing free radicals which causes damage to human system, hence lowers the risk of chronic diseases like cancer[39]. TPC, TFC, and antioxidant activity are the parameters which make quality tea and the assays should be applied for quality control of tea manufacturing. The comparison with the studies done on the different species of wild rosehips and *R. moschata* showed a significant good free radical scavenging activity in its dried samples. As per the literature review, wild rosehips from the Himalayan region of Kullu valley have not been studied till date. The study conducted on the species shows an overall good antioxidant property in its extract, therefore, having the potential to develop an enterprise of health beverages such as tea.

Conclusion

According to the Tea Association of the USA, about 84% of all tea consumed in the year 2018-19 in the USA was black tea, 15% was green Tea, and the small remaining amount was oolong, white and dark tea for various health benefits. Therefore, *Rosa moschata* pods which showed very good phenolic content, flavonoid content, and good antioxidant activities is recommended to be used as tea. Further, research is needed on the above aspect to assess all other nutraceutical aspects of the species for larger benefit and promotion as a successful enterprise. At the same time, it could be a good alternative source of livelihood to locals while conserving local flora.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

References

1 Anwar H, Hussain G and Mustafa I, Antioxidants from natural resources, in *Antioxidant in Foods and its Applications*, (Intech Open), 2018, 3-27.

2 Abbasi M A, Shahwar D, Wahab M and Saddiqui M F, Antibacterial and antioxidant activities of an ethnobotanically important plant *Sauromatum venosum* (Ait.) Schott. of District Kotli, Azad Jammu & Kashmir, *Pak J Bot*, 2011, **43**(1), 579-585.

3 Tiwari A K, Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy, *Curr Sci*, 2001, **81**(9), 1179-1187.

4 Sen A R and Mandal P K, Use of natural antioxidants in muscle foods and their benefits in human health: An overview, *Int J Meat Sci*, 2017, **7**(1), 1-5.

5 Shan S, Huang X, Shah M H and Abbasi A M, Evaluation of polyphenolics content and antioxidant activity in edible wild fruits, *Bio Med Res Int*, 2019, **2019**, 1-11.

6 Rawat S, Jugran A K, Bahukhandi A, Bahuguna A, Bhatt I D, *et al.*, Anti-oxidant and anti-microbial properties of some ethno-therapeutically important medicinal plants of Indian Himalayan Region, *Biotech*, 2016, **6**(2), 154.

7 Baba S A and Malik S A, Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume, *J Taibah Univ Sci*, 2015, **9**(4), 449–454.

8 Lobo V, Patil A, Phatak A and Chandra N, Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacogn Rev*, 2010, **4**(8), 118-126.

9 Bagchi K and Puri S, Free radicals and antioxidants in health and disease, *East Mediterranean Health J*, 1998, **4**(2), 350-360.

10 Dimitrios B, Source of natural phenolic antioxidants, *Trends Food Sci Tech*, 2006, **17**(9), 505-512.

11 Aoshima H, Hirata S and Ayabe S, Anti-oxidative and anti-hydrogen peroxide activities of various herbal teas, *Food Chem*, **103**(2), 2007, 617–622.

12 Loranty A, Rembialkowska E, Rosa E and Bennett R N, Identification, quantification and availability of carotenoids and chlorophylls in fruit, herb and medicinal teas, *J Food Compos Anal*, 2010, **23**(5), 432-444.

13 Ravikumar C, Review on herbal teas, *J Pharm Sci Res*, 2014, **6**(5), 236-238.

14 The International Plant Names Index, The Plant List, Available on: http://www.ipni.org/ipni/idPlantNameSearch.do?id=731884-1, accessed on 14th April 2020.

15 Wissemann V and Ritz C M, The genus Rosa (Rosoideae, Rosaceae) revisited: Molecular analysis of nrITS-1 and atp B-rbcL intergenic spacer (IGS) versus conventional taxonomy, *Bot J Linnean Soc*, 2005, **147**(3), 275-290.

16 Sharma B, Singh B, Dhyani D, Verma P K and Karthigeyan S, Fatty acid composition of wild growing rose species, *J Med Plant Res*, 2012, **6**(6), 1046-1049.

17 Demir F and Ozcan M, Chemical and technological properties of rose (*Rosa canina* L.) fruits grown wild in Turkey, *J Food Eng*, 2001, **47**(4), 333-336.

18 Al-Obaidi R S S and Sahib D H, Determination of antioxidants activity in Tea extract, *Am J Biochem*, 2015, **5**(3), 49-52.

19 Shashni S, Sharma S, Rathore S, Samant S S and Sundriyal R C, Traditional uses and potential to develop an enterprise of wild rose species *Rosa brunonii* syn *moschata* in Kullu district of North Western Himalaya, *J Non Timber Forest Prod*, 2019, **24**(2), 137-139.

20 Chowdhery H J and Wadhwa B M, *Flora of Himachal Pradesh: analysis*, vol I, (Botanical Survey of India, Calcutta), 1984.

21 Dhaliwal D S and Sharma M, Flora of Kullu district, Himachal Pradesh, (Bishen Singh Mahendra Pal Singh, Dehradun), 1999.

22 Frederick C, Wagner A and Morvillo N, Randomly amplified polymorphic DNA (RAPD) analysis of the musk roses (*Rosa moschata*), *Proc Fla State Hort Soc*, 2002, **115**, 117-119.

23 Ouerghemmi S, Sebei H, Siracusa L, Ruberto G, Saija A, *et al.*, Comparative study of phenolic composition and antioxidant activity of leaf extracts from three wild Rosa species grown in different Tunisia regions: *Rosa canina* L., *Rosa moschata* Herrm. and *Rosa sempervirens* L., *Ind Crops Prod*, 2016, **94**, 167-177.

24 Chandra S, Khan S, Avula B, Lata H, Yang M H, *et al.*, Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study, *Evid-Based Complement Altern Med*, 2014, **2014**, 1–9.

25 Genwali G R, Acharya P P and Rajbhandari M, Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity, *Nepal J Sci Tech*, 2013, **14**(1), 95-102.

26 Chang C, Yang M, Wen H and Chern J, Estimation of total flavonoids content in propolis by two complementary colorimetric methods, *J Food Drug Anal*, 2002, **10**(3), 178-182.

27 Brand-Williams W, Cuvelier M E and Berset C, Use of a free radical method to evaluate antioxidant activity, *LWT-Food Sci Technol*, 1995, **28**(1), 25-30.

28 Akter M S, Ahmed M and Eun J B, Solvent effects on antioxidant properties of persimmon (*Diospyros kaki* L. cv. *Daebong*) seeds, *Int J Food Sci Technol*, 2010, **45**(11), 2258-2264.

29 Arnao M B, Cano A and Acosta M, The hydrophilic and lipophilic contribution to total antioxidant activity, *Food Chem*, 2001, **73**(2), 239-244.

30 Benzie I F and Szeto Y, Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay, *J Agric Food Chem*, 1999, **47**(2), 633-636.

31 Roman I, Stănilă A and Stănilă S, Bioactive compounds and antioxidant activity of *Rosa canina* L. biotypes from spontaneous flora of Transylvania, *Chem Cent J*, 2013, **7**(1), 1-10.

32 Yoo K M, Lee C H, Lee H, Moon B and Lee C Y, Relative antioxidant and cytoprotective activities of common herbs, *Food Chem*, 2008, **106**(3), 929-936.

33 Fattahi S, Jamei R and Hosseini S S, Antioxidant and antiradical activities of *Rosa canina* and *Rosa pimpinellifolia* fruits from West Azerbaijan, *Iranian J Plant Physiol*, 2012, **2**(4), 523-529.

34 Yilmaz S O and Ercisli S, Antibacterial and antioxidant activity of fruits of some rose species from Turkey, *Roma Biotechnol Lett*, 2011, **16**, 6407-6411.

35 Demir N, Yildiz O, Alpaslan M and Hayaloglu A, Evaluation of volatiles, phenolic compounds and antioxidant activities of rosehip (*Rosa* L.) fruits in Turkey, *LWT Food Sci Technol*, 2014, **57**(1), 126-133.

36 Koczka N, Stefanovits-Bányai E and Ombodi A, Total polyphenol content and antioxidant capacity of rosehips of some Rosa species, *Medicines*, 2018, **5**(3), 1-10.

37 Nenadis N, Lazaridou O and Tsimidou M Z, Use of reference compounds in antioxidant activity assessment, *J Agric Food Chem*, 2007, **55**(14), 5452-5460.

38 Payne A C, Mazzer A, Clarkson G J and Taylor G, Antioxidant assays - consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves, *Food Sci Nutr*, 2013, **1**(6), 439-444.

39 Li X, Wu X and Huang L, Correlation between antioxidant activities and phenolic contents of radix *Angelicae sinensis* (Danggui), *Molecule*, 2009, **14**(12), 5349-5361.