



Assessment of total phenolics, flavonoids, and antioxidant properties within the genus *Cotoneaster* in Kashmir Himalayas

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Abstract

Cotoneaster, a member of the Rosaceae family comprises around 300 species globally and has attained a significant place in traditional medicine. Although polyphenols are known to be prevalent in most *Cotoneaster* species, very little is known about their specific content and antioxidant activity. Therefore, the current study aimed to evaluate the content of total phenolics, total flavonoids, and antioxidant activity of six *Cotoneaster* species viz, *Cotoneaster integerrimus*, *Cotoneaster affinis*, *Cotoneaster nummularia*, *Cotoneaster horizontalis*, *Cotoneaster rosea*, and *Cotoneaster microphylla* growing in Kashmir Himalaya. The folin-Ciocalteu method and aluminium chloride method were used to evaluate the total phenolics and total flavonoids respectively. Also, the antioxidant properties of the methanolic extracts were assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Reducing power assays. The results revealed noteworthy antioxidant activity and total phenolic content. The fruit extracts of *C. rosea* and *C. nummularia* had the highest content of phenolic and flavonoids (297.05 0.63 mg/g GAE and 30.47 0.22 mg/g QE, respectively). The highest antioxidant activity observed by the DPPH was found in the fruit extract of *C. rosea* (IC₅₀ = 3.45 ± 0.01). Moreover, Antioxidant activity and total phenolic content were shown to be significantly correlated. This study underscores the potential therapeutic value of these *Cotoneaster* species and positions them as a valuable source of potent natural antioxidants for pharmaceutical and nutraceutical applications. However, further research is required to evaluate the toxicity and other biological properties of these crude extracts, along with the identification of different compounds present within them.

Keywords Methanolic extracts · Polyphenols · *Cotoneaster* · DPPH · Natural antioxidants

Introduction

Free radicals are highly reactive and unstable chemical entities with unpaired electrons. Free radicals of significant biological importance are oxygen-reactive species, including carboxy radicals, hydroxy radicals, superoxide radicals, hydrogen peroxide, and singlet oxygen (Schöneich 1999). These free radicals are formed in the human body and can intrude into important macromolecules, thereby causing cell damage, heart disease, cancer, and aging (Finkel and Holbrook 2000). In addition to endogenous defensive mechanisms, nutritional antioxidants, primarily plant phenols, play a crucial role in shielding live cells from pro-oxidants

(Pandey and Rizvi 2009). As per the reports, synthetic antioxidants, including butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are harmful to humans (Kahl and Kappus 1993). People seek out natural antioxidants used in pharmaceuticals, food, and cosmetics as alternatives to synthetic antioxidants that may be hazardous and toxic (Liu and Yang 2018). The use of natural antioxidants can aid in improving the stability of meals, nutrients, and medications, while also enhancing the body's capacity to fight inflammation, allergies, and tumors due to the polyphenolic properties of these antioxidants (Moure et al. 2001).

Antioxidants are chemical substances that occur naturally in foods and the human body (Mititelu et al. 2020; Sharifi-Rad et al. 2020). These chemicals are critical in reducing cell damage brought on by oxidative degradation because of free radical production (Salehi et al. 2020). In recent years, scientists have begun to investigate the potential of plants as sources of antioxidants and substances that have the ability to treat diseases with few adverse consequences (Teo 2021).

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Sarkar et al. (2022) discussed the potential of plant-derived agents in treating various diseases and the use of phenolic-rich extracts as a substitute for synthetic antioxidants, which are becoming increasingly popular and approved over synthetic antioxidants because they appear less harmful and more effective. Among the many plants that have attracted scientists' curiosity, the *Cotoneaster* genus appears to be a potential candidate for further study.

One of the most commercially significant plant families in the world is the Rosaceae family, which includes the genus *Cotoneaster* and consists of approximately 300 species worldwide, including mostly woody (shrubs, rarely small trees) species (Popoviciu et al. 2020), with the highest concentration of taxa in the Himalayas and West China (Bartish et al. 2001). Since *Cotoneaster* produces extracts that are used as antitumor, expectorant, antioxidant, hepatoprotective, diuretic, cardioprotective, antiviral, and antispasmodic agents, it has been used in traditional medicine (Cakilcioglu and Turkoglu 2010). Some of the species in this study have also been used in traditional and local medicines. The *Cotoneaster affinis* fruits are used to improve digestion and cure abdominal pain in Pakistan (Shah and Hussain 2012). The leaves and fruits of *Cotoneaster integerrimus* are used to treat diabetes mellitus, coughs, fevers, and digestive issues (Zengin et al. 2014). The *Cotoneaster nummularia* manna fruits are used to treat neonatal jaundice in Iran (Fakhri et al. 2016). The plant parts of *Cotoneaster microphyllus* are used to treat irregular menstruation, bile malfunctioning diseases, expectorant, astringent, stomach-ache, haemostatic, and a remedy for cuts, wounds, and diarrhoea (Haq et al. 2011; Swati et al. 2018). There may not always be scientific support for many traditional practices, which are sometimes founded on cultural beliefs and historical events. The bioactive substances and possible health advantages linked to the use of *Cotoneaster* species must therefore be validated and understood through additional research. The presence of considerable content of total phenolics in the plant species may significantly contribute to the antioxidant properties and thus can serve as a rich source of natural antioxidants for pharmaceutical and nutraceutical applications. Although polyphenols constitute the majority of *Cotoneaster* species, very little is known about their presence and antioxidant activity (Zengin et al. 2014). According to Zengin et al. (2014), plant tissues comprising leaves and fruits from *Cotoneaster* species, other than those under study, can be predicted to have a considerably higher phenolic content. Therefore, the selection criteria of the species included in this study were influenced by a combination of factors including documented historical uses, and the existence of preliminary scientific evidence that suggests bioactivity. Our study does not rely exclusively on traditional practices; rather it adheres to modern pharmacological bioprospecting concepts, which emphasizes the significance of investigating

many plant species for their unrealized medicinal potential. Our approach could be biased toward bioprospecting, but it aims to strike a balance between traditional wisdom and cutting-edge scientific investigations. Additionally, some species mentioned in this study have not yet been studied. Furthermore, to the best of our knowledge, this is the first study of its kind on the genus *Cotoneaster* from the Kashmir region of India. Based on the above knowledge gap, the goal of this study was to evaluate and compare the content of total phenolics, total flavonoids, and antioxidant activity of the leaves and fruits of six *Cotoneaster* species viz, *Cotoneaster integerrimus*, *Cotoneaster affinis*, *Cotoneaster nummularia*, *Cotoneaster horizontalis*, *Cotoneaster rosea*, and *Cotoneaster microphylla*. We believe this study will provide scientific insights that corroborate, enhance, or reinterpret their traditional significance and ultimately, will contribute to a broader understanding of the bioactive potential of these *Cotoneaster* species and their potential applications in modern health contexts.

Materials and methods

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), Potassium ferricyanide, Quercetin, Gallic acid, FC reagent, Sodium carbonate, Phosphate buffer, Aluminium chloride, Sodium acetate, Methanol, Trichloro acetic acid, Ferric chloride, Ascorbic acid.

Plant material

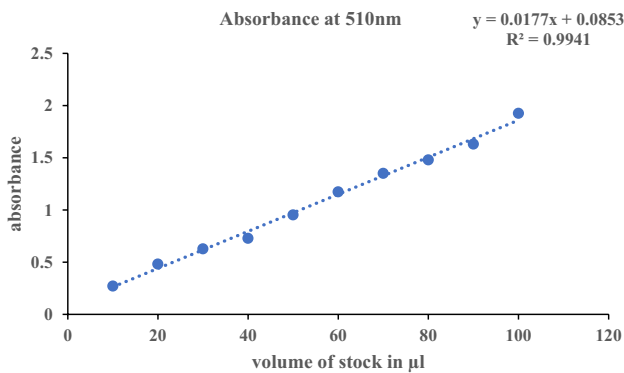
Leaves and fruits of six species of Genus *Cotoneaster* viz, *C. integerrimus*, *C. affinis*, *C. nummularia*, *C. horizontalis*, *C. rosea*, and *C. microphylla* were collected from the different localities of Kashmir Himalayas in September 2022. The different localities and their geo-coordinates are given in Table 1. The identification and validation of the target species were done at the Center of Plant Taxonomy, Department of Botany, University of Kashmir. The specimens of each species were submitted to the KASH herbarium of the Department of Botany.

Preparation of the plant extracts

The collected fruits and leaves of all six species were washed and cleaned with a tissue towel. Pyrenes of mature pomes of various individuals from all six species were manually removed and the pulp of the fruit was ground in a mortar using liquid nitrogen to get a fine powder whereas the collected leaves of all species were dried in the shade and were grounded to a fine powder using a mortar and pestle. One

Table 1 The different localities with their geo-coordinates from where the species were collected

S No	Species	Locality/Location	Geo-coordinate	Altitude
1	<i>C. integerrimus</i>	Arzan sahab Cherhar Sopore	34° 22' 34.72" N 74° 28' 40.21" E	2076 m
2	<i>C. affinis</i>	Overa wildlife Sanctuary	33° 56' 30.27" N 75° 16' 11.87" E	2137 m
3	<i>C. nummularia</i>	Chorwan gurez	34° 38' 19.22" N 74° 52' 38.39" E	2817 m
4	<i>C. horizontalis</i>	Markoot Gurez	34° 37' 54.19" N 74° 51' 26.12" E	2475 m
5	<i>C. rosea</i>	Wanpora Gurez	34° 37' 50.80" N 74° 48' 15.24" E	2465 m
6	<i>C. microphylla</i>	Rampora – Rajpora road Sopore	34° 22' 43.59" N 74° 30' 32.77" E	1882 m

**Fig. 1** Quercetin standard curve

gram (1 g) of pulverized material was extracted in 20 ml of methanol, left overnight on a shaker, and centrifuged for 10 min at 10,000 rpm. The collected supernatant was filtered through Whatman filter paper (No. 1) and used in different biochemical assays.

Estimation of total flavonoid content (TFC)

TFC was calculated using an aluminium chloride method (Bag et al. 2015). Briefly, 50 μ L of the plant extract was taken, and the volume was made up to 1 ml using methanol. To this 100 μ L of 10% aluminum chloride was added and shaken well. After 5 min 100 μ L of sodium acetate (1 M) and distilled water (2.8 ml) were added and shaken thoroughly. The mixture was then incubated at room temperature in the dark for 1 h. The absorbance was measured at 510 nm using a UV-1900 I spectrophotometer. Without the extract, 1 ml of methanol and all the reagents were used as blank. Quercetin was used as a standard. A standard calibration (Fig. 1) curve was created using quercetin solutions (1 mg/ml) at various

concentrations. mg equivalent of quercetin (QE) per gram of extract was used to determine the overall flavonoid content. The experiment was performed in triplicate.

Estimation of total phenolic content (TPC)

The TPC of the methanolic extracts of plant parts was calculated using the Folin-Ciocalteu method (Singleton and Rossi 1965). Briefly, 1 ml of plant extract was taken, and 5 ml of FC reagent (2 N, 1 FC:10 distilled water) was added and mixed thoroughly. Subsequently, 4 ml of 20% sodium carbonate was added and thoroughly mixed for 5 min. After one hour of incubation at room temperature in the dark, the absorbance of the mixture was measured at 760 nm. For comparison, gallic acid was selected, and the data were expressed as the milligram equivalents of gallic acid per gram of extract. The experiment was performed in triplicate.

Antioxidant properties

DPPH radical scavenging activity

DPPH assay was performed to evaluate the potential of the plant extracts to scavenge free radicals (Chu et al. 2000). The DPPH solution (0.004%) was prepared using methanol. Test tubes were filled with various concentrations (1.5–30 μ l) of the plant extract and made up to 1 ml using methanol, followed by the addition of 2 ml of DPPH solution. The test tubes were covered with aluminum foil to shield them from light and then left at room temperature for 30 min in the dark. Using a spectrophotometer (UV-1900 I), the absorbance was measured at 517 nm following incubation. DPPH was used as a control, without any extract, and methanol was taken as a blank. The following equation was used to determine the percentage of DPPH free radical-scavenging activity:

$$\%age\ scavenging\ activity = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} \times 100$$

All concentrations in the experiment were measured in triplicate. Furthermore, the experiment was performed in triplicate. A calibration curve was plotted for percentage scavenging activity, providing the equation $y = mx + c$ from which the IC50 value was calculated.

Reducing power assay

The reducing power of the samples was tested according to the method of (Dorman and Hiltunen 2004). Plant extracts and ascorbic acid standard of different concentrations were made up to 1 ml using methanol, and mixed well with potassium ferricyanide (2.5 ml, 1%) and phosphate buffer (2.5 ml, P.H. 6.6, 0.2 M). The mixture was kept at 50°C in a water bath for 20 min. Trichloroacetic acid (2.5 ml, 10%) was then added. The mixture was centrifuged at 3000 rpm for 5 min. The supernatant (2.5 ml) was combined with 2.5 ml of distilled water and 0.5 ml of ferric chloride, and absorbance was measured at 700 nm. Similar steps were performed to prepare the controls but without the extracts. The increase in the reducing power of the mixture was denoted by the increase in absorbance.

Statistical analysis

Execution of the analysis of variance (ANOVA) was performed and the significant differences between the mean values were calculated by Duncan's post-hoc analysis ($P < 0.05$) using SPSS v23.0 (IBM SPSS Statistics 2015). The correlation between variables was analysed using 'R' software (R Core Team 2022).

Results and discussion

Total phenolics

The TPC in methanolic extracts of fruits and leaves of different species was calculated using the standard curve equation obtained from Fig. 2. ($y = 0.0071x + 0.2446$, where $R^2 = 0.9987$) using gallic acid and expressed as milligram equivalents of gallic acid per gram of extract. The results of the TPC are shown in Table 2. The TPC analysis revealed significant variations between different species of *Cotoneaster* both in terms of leaf and fruit. In terms of fruit extracts the highest TPC was observed in *C. rosea* (297.05 ± 0.63^f mg/g GAE), followed by *C. affinis* (241.46 ± 1.06^e mg/g GAE). The lowest TPC was recorded for the *C. integerrimus*

(97.14 ± 0.80^a mg/g GAE). With respect to leaves, the highest TPC was found in *C. integerrimus* (277.14 ± 0.58^f mg/g GAE), followed by *C. microphylla* (270.47 ± 0.37^e mg/g GAE). The lowest TPC content was found in the leaves of *C. horizontalis* (22.68 ± 0.21^a mg/g GAE). This is in accordance with the study of Yoo et al. (2019) for *C. wilsonii* with estimates of TPC = 214.65 ± 2.43 for methanolic stem extracts, and 220.95 ± 0.50 for methanolic leaf extracts, whereas for the fruit methanolic extracts, the TPC was relatively low (60.71 ± 2.03). Similarly, Zengin et al. (2014) reported different polarity extracts ranging from 8.11 to 266.39 mgGAE/g for *C. nummularia*. The antioxidant capabilities of any plant are directly correlated with its phenolic content. Phenolic compounds can scavenge free radicals, and operate as hydrogen donors, and reducing agents (Wojdyło et al. 2007). The presence of considerable content of total phenolics in the leaves and fruits of these species may significantly contribute to the antioxidant properties and thus can serve as a rich source of natural antioxidants for pharmaceutical and nutraceutical applications. Additionally, the species *C. integerrimus*, *C. microphylla*, *C. nummularia*, and *C. affinis* may have been utilized in conventional medications as a result of these properties. The total phenolic content of the various species tested in this study varied, which may be due to variances in the environmental conditions and genetic makeup of the species (Doshi et al. 2006).

Total flavonoids

The TFC in methanolic extracts of fruits and leaves of different species was calculated using the standard curve equation obtained from Fig. 1. ($y = 0.0177x + 0.0853$, where $R^2 = 0.9941$) using Quercetin and expressed as milligram

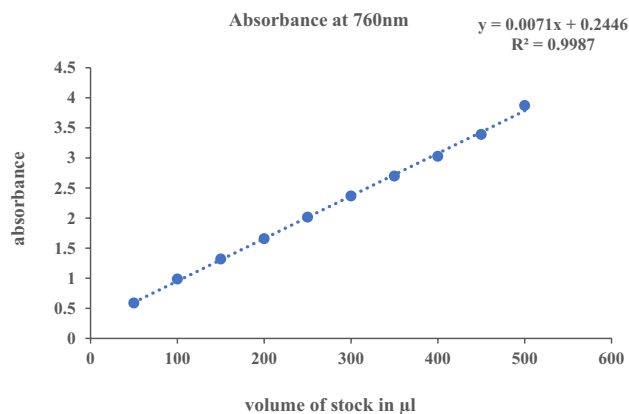


Fig. 2 Gallic acid standard curve

Table 2 Total phenolic and total flavonoid content of fruit and leaf of different *Cotoneaster* species

Species	Leaf		Fruit	
	Total phenolic content	Total flavonoid content	Total phenolic content	Total flavonoid content
<i>C. integerrimus</i>	277.14 ± 0.58 ^f	9.51 ± 0.14 ^d	97.14 ± 0.80 ^a	13.37 ± 0.20 ^c
<i>C. affinis</i>	238.13 ± 0.29 ^e	7.12 ± 0.22 ^c	241.46 ± 1.06 ^e	5.63 ± 0.22 ^b
<i>C. nummularia</i>	259.16 ± 0.45 ^d	7.25 ± 0.34 ^c	154.89 ± 0.49 ^b	30.47 ± 0.22 ^d
<i>C. horizontalis</i>	22.68 ± 0.21 ^a	5.51 ± 0.25 ^b	181.55 ± 0.43 ^d	0.35 ± 0.22 ^a
<i>C. rosea</i>	124.09 ± 0.49 ^b	2.31 ± 0.26 ^a	297.05 ± 0.63 ^f	0.32 ± 0.22 ^a
<i>C. microphylla</i>	270.47 ± 0.37 ^e	9.94 ± 0.25 ^d	170.05 ± 0.37 ^c	5.55 ± 0.19 ^b

TPC total phenolic content, expressed as gallic acid equivalents, TFC total flavonoid content, expressed as Quercetin equivalents

All values are presented as the means of triplicates ± standard deviation (SD), different letters within the same rows indicate significant differences at $p < 0.05$ by Duncan's multiple range post hoc test

equivalents of quercetin per gram of extract. The results of the TFC are shown in Table 2. In the context of fruits, the highest TFC was observed in *C. nummularia* (30.47 ± 0.22^d mg/g QE), followed by *C. integerrimus* (13.37 ± 0.20^c mg/g QE). The lowest content was recorded for *C. rosea* and *C. horizontalis* (0.32 ± 0.22^a and 0.35 ± 0.22^a mg/g QE respectively). In terms of leaf extracts the highest TFC content was noted in *C. microphylla* (9.94 ± 0.25^d mg/g QE), followed by *C. integerrimus* (9.51 ± 0.14^d mg/g QE). The lowest content was recorded for *C. rosea* (2.31 ± 0.26^a mg/g QE). We found differences between the results of our TFC assessment and the earlier findings that had been published in the literature (Popoviciu et al. 2020). In particular, the TFC values obtained from *C. horizontalis* fruit extracts in our investigation were significantly lower (0.35 ± 0.22^a mg/g QE) than Popoviciu's findings, who recorded a TFC range of 4.457–10.94 mg/g for the *C. horizontalis*. (Mohamed et al. 2012) also noted a TFC value of 6.8 mg/g for the same species. However, the values for the *C. microphyllus* (5.55 ± 0.19^b mg/g QE) were in accordance with the findings of Popoviciu who reported a TFC range of 3.44 – 10.33 mg/g for the same species. These differences in TFC between our study and the previous reports could potentially be attributed to a range of factors, such as environmental conditions, variation in sample collection, and analysis techniques. In addition, it is worth mentioning that there is a dearth of comprehensive literature available pertaining to these *Cotoneaster* species underscoring the need for meticulous investigation.

Antioxidant activity

The antioxidant capacities of methanol extracts of the fruits and leaves of *Cotoneaster* species were evaluated using a free radical scavenging assay (DPPH) and a reducing power assay. The IC₅₀ values were used to determine the free radical scavenging activities. The extract concentrations of both leaves and fruits required to neutralize 50% of the DPPH radicals are expressed in terms of IC₅₀

Table 3 Antioxidant activity of fruit and leaf of different *Cotoneaster* species in terms of IC₅₀ value calculated by DPPH assay

S no.	Species	IC 50 value	
		Fruit	Leaf
01	<i>C. integerrimus</i>	18.15 ± 0.13 ^f	4.15 ± 0.02 ^a
02	<i>C. affinis</i>	4.48 ± 0.1 ^b	6.63 ± 0.05 ^c
03	<i>C. nummularia</i>	5.94 ± 0.01 ^d	5.17 ± 0.10 ^b
04	<i>C. horizontalis</i>	7.02 ± 0.04 ^e	30.73 ± 0.07 ^e
05	<i>C. rosea</i>	3.45 ± 0.01 ^a	12.61 ± 0.02 ^d
06	<i>C. microphylla</i>	5.47 ± 0.03 ^c	5.01 ± 0.32 ^b
07	Ascorbic acid	2.32 ± 0.02	

All values are presented as the means of triplicates ± standard deviation (SD)

values and are listed in Table 3. The extracts showed a concentration-dependent increase in their ability to scavenge free radicals. Ascorbic acid was used as standard. The IC₅₀ value of ascorbic acid was 2.32 ± 0.02. In the context of fruits, the highest radical scavenging activity was noted in *C. rosea* with an IC₅₀ value of 3.45 ± 0.01^a, followed by *C. affinis* (IC₅₀ 4.48 ± 0.1^b). the lowest radical scavenging activity was noted in *C. integerrimus* (IC₅₀ 18.15 ± 0.13^f). In the case of leaf extracts the highest radical scavenging activity was observed in *C. integerrimus* with an IC₅₀ of 4.15 ± 0.02^a, followed by *C. microphylla* (IC₅₀ 5.01 ± 0.32^b), and *C. nummularia* (IC₅₀ 5.17 ± 0.10^b). the lowest radical scavenging activity in leaf extracts was noted in *C. horizontalis* with an IC₅₀ of 30.73 ± 0.07^e. The ability of the isolated fruit and leaf samples to scavenge DPPH free radicals at various concentrations was remarkable. For most of the species tested, the IC₅₀ value for fruits and leaves was found to be less than 10, indicating a remarkably high level of antioxidant activity. Notably, the fruits of *C. rosea*, leaves of *C. integerrimus*, and leaves of *C. affinis* showed almost the same results as the ascorbic acid standard. The results of this study suggest that these

species have a high level of antioxidant potency, indicating their potential use as sources of natural antioxidants. The discrepancies in IC₅₀ values between these species show the unique antioxidant chemical profiles of each species and most of the antioxidant activity in plants is because of the phenols (Mansouri et al. 2005). Remarkably, our findings align well with the overall pattern of total phenolic content observed in these *Cotoneaster* species and the total phenolic content suggests that phenolic compounds might indeed be the principal contributors to the observed antioxidant potential.

Results from other previous studies seem to be difficult to contrast because of the other factors used during the experiments. However, the DDPH test was used to examine the antioxidant activity of 70% methanol extract fractions of three species of *Cotoneaster's* leaves cultivated in Poland (Kicel et al. 2018). According to the authors, the IC₅₀ values ranged from 3.19 to 27.88 µg/mL for the *C.*

bullatus ethyl acetate fraction and *C. integerrimus* water residue thereby showing a strong scavenging effect. In addition, (Krzemińska et al. 2022) investigated the antioxidant potential of several leaf extracts from *C. hsingshangensis* and *C. hissarius* using DPPH assay. The *C. hsingshangensis* ethyl acetate fraction showed the highest DPPH scavenging activity (IC₅₀ = 2.08 ± 0.03 µg/mL), followed by the butanol fraction (IC₅₀ = 3.43 ± 0.02 µg/mL) and the diethyl ether fraction (IC₅₀ = 4.15 ± 0.05 µg/mL). The water fraction exhibited the lowest activity (IC₅₀ = 32.37 ± 0.19 µg/mL) and crude extract of *C. hissarius* (IC₅₀ = 21.73 ± 0.13 µg/mL).

From the reducing power assay, as shown in Figs. 3 and 4, as the sample concentration increased, the absorbance increased for both the leaves and fruits, indicating their antioxidant activity. The highest reducing power was found in the fruits of *C. rosea*, followed by those of *C. affinis*. Their effect was found to be higher than that of ascorbic acid

Fig. 3 Comparison of Reducing power of ascorbic acid and the fruit of different *Cotoneaster* species. Sp 1 = *C. integerrimus*; Sp 2 = *C. affinis*; Sp 3 = *C. nummularia*; Sp 4 = *C. horizontalis*; Sp 5 = *C. rosea*; Sp 6 = *C. microphylla*. Results expressed as the mean ± standard deviation (n=3)

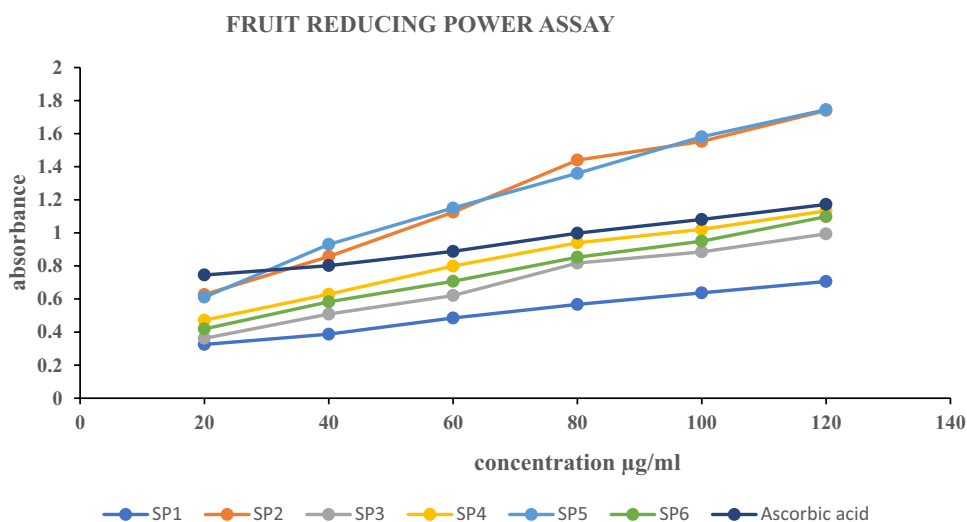
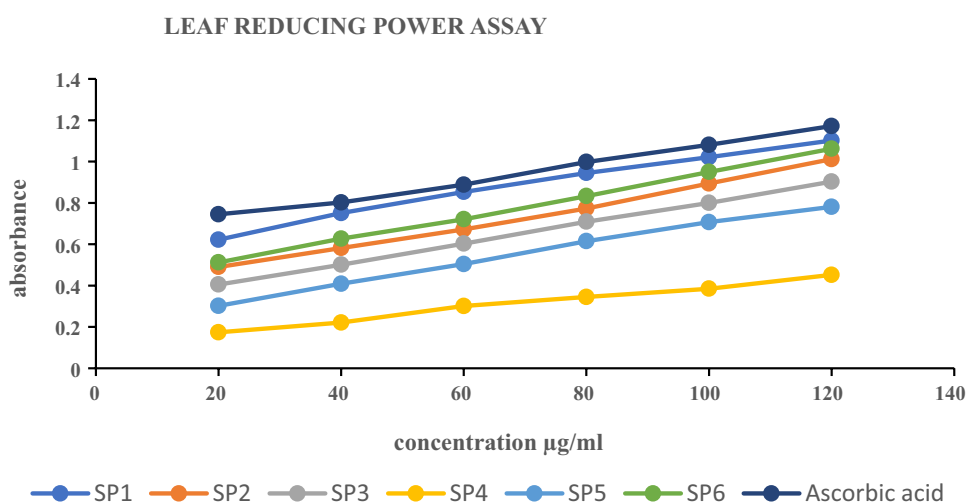


Fig. 4 Comparison of Reducing power of ascorbic acid and the leaf of different *Cotoneaster* species. Sp 1 = *C. integerrimus*; Sp 2 = *C. affinis*; Sp 3 = *C. nummularia*; Sp 4 = *C. horizontalis*; Sp 5 = *C. rosea*; Sp 6 = *C. microphylla*. Results expressed as the mean ± standard deviation (n=3)

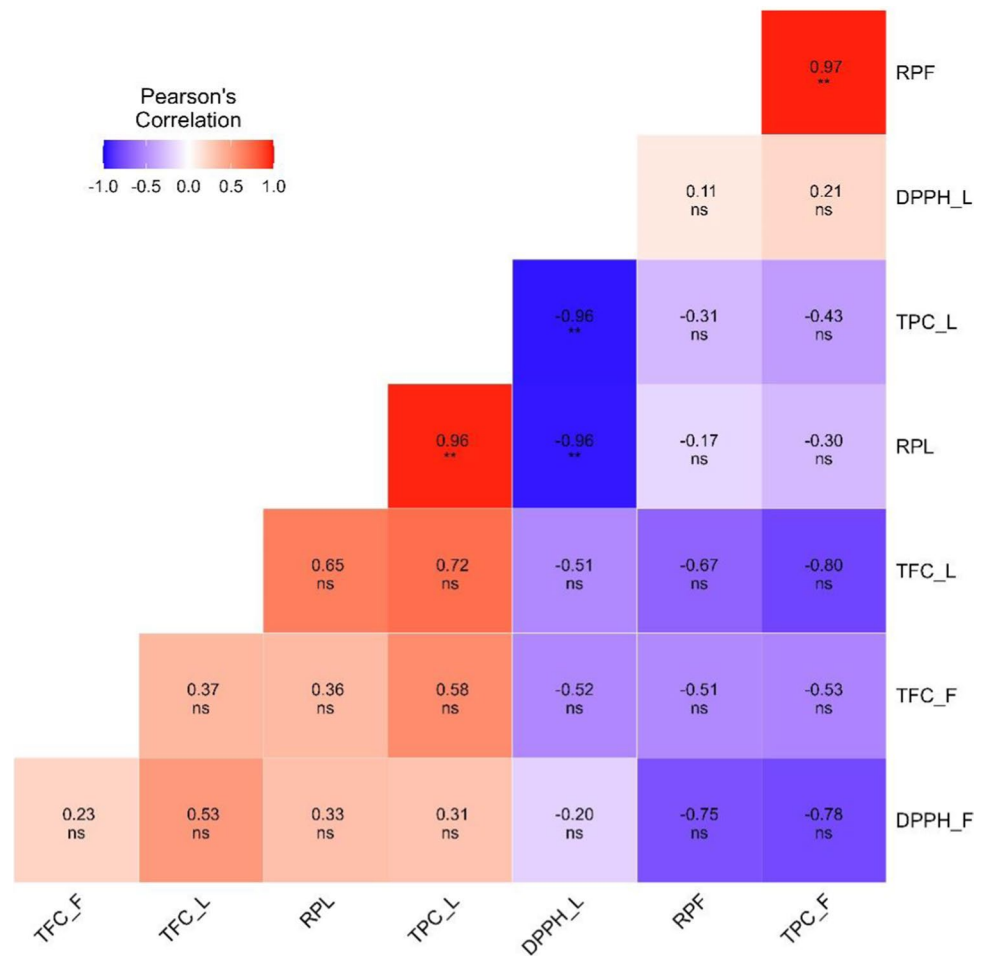


acid used as a standard when treated from 40 to 120 µg/ml, whereas the lowest was found in the leaf samples of *C. horizontalis*. Yoo et al. (2019) studied different parts of *C. wilsonii* and obtained similar results for the tested extracts and the positive control ascorbic acid. Antioxidant activity and reducing power are related, and reducing power may be a useful indicator of antioxidant activity. Reducing power compounds donate electrons to oxidize intermediary compounds of lipid peroxidation processes, in order for them to serve as both primary and secondary antioxidants, this helps to prevent damage to cells and tissues caused by oxidative stress (Chanda and Dave 2009).

Linear regression analysis ($P < 0.01$) was conducted between the TPC, TFC, reducing power, and DPPH assays for all tested fruit and leaf samples. As shown in Fig. 5, the antioxidant activities of the tested species showed a strong correlation with TPC levels. The phenols and flavonoids containing hydrogen-donating groups cause the methanolic DPPH solution to get reduced as a result of the generation of non-radicals (Mensor et al. 2001). However, this dependence was not found in the case of TFC, which was probably

caused by the relatively lower amount of TFC. The results of the DPPH and reducing power assays were the same. The DPPH and reducing power assays also showed a strong correlation with each other, which implies that *Cotoneaster* extracts might have the potential to offer antioxidant benefits across different experimental approaches. They use both fundamental reaction pathways and function as direct quenchers of ROS, as well as reducing agents. Also, it might be said that the amount of TPC reflected in GAE is a significant indicator of antioxidant capacity. This could be used to screen plant extracts as natural sources of antioxidants. However, it is noteworthy that the negative signs in the relation between DPPH and TPC levels are because the data of the DPPH assays (radical scavenging activity) have been taken in terms of IC₅₀ value. The lower IC₅₀ value indicates higher radical scavenging activity and vice-versa. Our findings support those of Olszewska et al. (2010) and Sravani and Padma (2011). However, it is necessary to conduct a more thorough investigation of the compounds contained in *Cotoneaster* samples and their chemical nature.

Fig. 5 Correlation map analysis among TPC (total phenolic content), TFC (total flavonoid content), DPPH, and Reducing power assay. The letter F and L on the map stands for fruit and leaf respectively



ns $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$

Conclusion

This study demonstrates the TPC, TFC, and antioxidant activities of six *Cotoneaster* species growing in the Kashmir Himalaya. Most of the samples showed a significantly high TPC and antioxidant activity. The fruits of *C. rosea* and leaves of *C. integerrimus* showed a remarkable high TPC and antioxidant activity. The highest TFC values were shown by the fruits of *C. nummularia*. The antioxidant activity of leaves and fruits positively correlate with their TPC values. The TPC, TFC, and antioxidant activity of the various species tested in this study varied, which may be due to variances in the environmental conditions and genetic makeup of the species. The remarkable antioxidant activity of these species lends credence to the notion that their products could be valuable sources of potent natural antioxidants that can be used in the diet, medications, cosmetics, and other applications where antioxidants are needed. However, further research is required to evaluate the crude extracts of these species and identify different compounds present in them. Additionally, their toxicity and other biological properties for potential use in the pharmaceutical and nutraceutical sectors need to be evaluated.

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Data availability The data will be available on request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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