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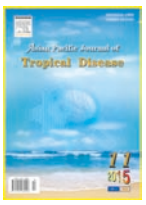

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Evaluation and comparison of polyphenols and bioactivities of wild edible fruits of North-West Himalaya, India

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ABSTRACT

Objective: To evaluate and compare the polyphenol contents, antioxidant, anti-elastase, anti-collagenase, anti-tyrosinase and anti-inflammatory activities of 13 wild edible fruits [*Pyracantha crenulata, Berberis asiatica* (*B. asiatica*), *Ficus subincisa* (*F. subincisa*), *Morus serrata, Ziziphus nummularia, Leea asiatica* (*L. asiatica*), *Dendrobenthamia capitata, Ziziphus mauritiana, Prunus cerasoides, Ampelocissus latifolia* (*A. latifolia*), *Vitis jacquemontii, Morus alba* and *Grewia optiva*] of North-West Himalayan Region of India.

Methods: Fruits extracts were prepared with 80% aqueous acetone and evaluated for total phenolic contents (TPC) and total flavonoid contents (TFC). Free radical scavenging activities [against 1,1-diphenyl-2-picryl-hydrazyl, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), linoleate hydroperoxyl and superoxide radicals], ferric reducing ability, ferrous metal chelating capacity, anti-elastase, anti-collagenase, anti-tyrosinase and anti-inflammatory activities were determined by using various *in vitro* assays.

Results: TPC varied from 58.83 to 4 496.39 mg gallic acid equivalents/100 g fruit weight (FW), being highest in *A. latifolia* and lowest in *F. subincisa*. TFC ranged from 108.00 to 1 963.75 mg catechin equivalents/100 g FW, standing highest in *L. asiatica* and lowest in *Prunus cerasoides*. *A. latifolia* and *L. asiatica* possessed the highest antioxidant activities while *B. asiatica* and *L. asiatica* owned uppermost anti-elastase and anti-collagenase activities, respectively. *B. asiatica* revealed the highest anti-tyrosinase activity and *F. subincisa* demonstrated the highest anti-inflammatory activity. The present study revealed differential contribution of TPC and TFC in various antioxidant activities. However, no obvious relationship was visible between anti-elastase/anti-collagenase/anti-tyrosinase/anti-inflammatory activities and TPC/TFC, suggesting the role of individual or combination of specific phenolics and flavonoids.

Conclusions: The abilities of Himalayan wild edible fruits to scavenge a variety of free radicals, inhibit enzymes causing skin-aging and skin-darkening are highly appreciable, suggesting their possible utilization for the development of effective formulations for general health maintenance and anti-aging, skin-whitening cosmetics.

1. Introduction

Increased vegetables and fruits consumptions are well recognized for their associations with a lower risk of a number of chronic diseases, such as coronary heart diseases, cancers, inflammation, immune dysfunction and diabetes mellitus[1]. As a primary food source, fruits and vegetables provide life-sustaining nutrients including minerals, vitamins, phenolics and flavonoids. Polyphenols from commercially cultivated fruits such as apple, berries, banana, grapes, mango, oranges, pomegranate, papaya, pear and pineapple have been shown to possess many health boosting properties including super antioxidant, anti-cancer, anti-inflammatory and anti-microbial *in vitro*[2-5]. Besides commercial fruits, wild edible fruits are gaining immense global interest from researchers owing to their higher polyphenol contents and admirable antioxidant activities[6-11].

The Indian Himalayan Region owns approximately 675 wild edible plant species, out of which 340 were exclusively reported in Uttarakhand[12]. Of these, wild edible fruits are of great interest due to their nutritional values, vitamins, mineral contents and delicious taste. They have served as dietary staples and medicines for thousands of years, particularly in the tribal and rural areas of Uttarakhand[13]. Limited studies on wild edible fruits of Uttarakhand have shown presence of abundant polyphenol contents and antioxidant, anti-microbial, and anti-proliferative activities[9-11,14]. There are a lot of wild edible fruits which are still under exploration regardless of their nutritional values[13]. Therefore, extending exhaustive analysis of their various biological activities including antioxidant, anti-elastase, anti-collagenase, anti-tyrosinase and anti-inflammatory activities would be of immense significance which

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would not only encourage the conservation of these species but also give an impetus to their trade. In this regard, *Pyracantha crenulata* (*P. crenulata*), *Berberis asiatica* (*B. asiatica*), *Ficus subincisa* (*F. subincisa*), *Morus serrata* (*M. serrata*), *Ziziphus nummularia* (*Z. nummularia*), *Leea asiatica* (*L. asiatica*), *Dendrobenthamia capitata* (*D. capitata*), *Ziziphus mauritiana* (*Z. mauritiana*), *Prunus cerasoides* (*P. cerasoides*), *Ampelocissus latifolia* (*A. latifolia*), *Vitis jacquemontii* (*V. jacquemontii*), *Morus alba* (*M. alba*) and *Grewia optiva* (*G. optiva*) are some of the few common wild edible fruits consumed by the local population as food source and medicine, but their polyphenol contents and associated activities are not yet investigated thoroughly. Hence, the present study aimed to evaluate and compare the total polyphenols and their antioxidant, anti-elastase, anti-collagenase, anti-tyrosinase and anti-inflammatory activities of aforementioned fruits of Uttarakhand, India.

2. Materials and methods

All the chemicals were of analytical grade and more than 99% pure. 1,1-diphenyl-2-picrylhydrazyl (DPPH), catechin, nicotinamide adenine dinucleotide, phenyl methosulfate, nitro blue tetrazolium, β-carotene, linoleic acid, ferrozine, kojic acid, N-succinyl-Ala-Ala-Ala-p-nitroanilide, N-[3-(2-furyl)acryloyl]-leu-gly-pro-ala, 3-4-dihydroxy-L-phenylalanine (L-DOPA), epigallocatechin gallate (EGCG) and porcine pancreatic elastase (EC 3.4.21.36), *Clostridium histolyticum* collagenase (EC 3.4.24.3) and mushroom tyrosinase (EC 1.14.18.1) were procured from Sigma-Aldrich (St Louis, MO, USA). 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was obtained from Calbiochem, Merck Pvt. Ltd. (Darmstadt, Germany). Other chemicals and reagents were purchased from HiMedia Pvt. Ltd. (Mumbai, India).

2.1. Collection, identification and authentication of fruits

Ripened fruit samples (along with small twig containing leaves) were harvested from various hilly locations confined to Uttarakhand, India through out the year with reference to their fruiting times (Table 1). Fresh fruits were brought to the laboratory and cleaned under running tap water and kept at -20 °C till use within one month. The herbariums of fruit samples were deposited to Botanical Survey of India, Dehradun, India, for botanical identification and authentication.

2.2. Preparation of fruit extracts and evaluation of total phenolic contents (TPC) and total flavonoid contents (TFC)

Fruit extracts were prepared with 80% aqueous acetone as solvent[9]. TPCs were determined by Folin-Ciocalteau method[15]. Fruit extracts (1 mL) were incubated with Folin-Ciocalteau reagent (0.2 mol/L, 2.5 mL) at room temperature for 5 min. Thereafter, sodium carbonate solution (75 g/L in water, 2 mL) was added and further incubated for 2 h. Absorbance of reaction mixture was measured by using UV-Visible spectrophotometer (Systronics, India, Model No. 119) at 760 nm against a water control. A standard calibration curve was plotted by using gallic acid (0.2 mg/mL). The TPCs were expressed as mg gallic acid equivalent (GAE)/100 g fruit weight (FW).

TFCs of fruit extracts were determined according to methods described by Chang *et al*.[16]. Diluted extract (0.6 mL) was incubated with sodium nitrite solution (0.3 mL, 5%) for 5 min at room temperature. Afterwards, aluminium trichloride solution (0.6 mL, 10%) was added and incubated further for 5 min at room temperature. Absorbance was measured at 510 nm against water blank and TFC was expressed as mg catechin equivalents (CE)/100 g FW by using catechin (0.5 mg/mL) as standard.

2.3. Antioxidant activity

Antioxidant activities of fruit extracts were evaluated with respect to free radical scavenging activities such as DPPH radical scavenging activity, $ABTS^+$ radical scavenging activity, superoxide radicals scavenging activity, linoleate hydroperoxide radical scavenging activity, ferric reducing activity and ferrous metal chelating activity by using the protocols mentioned by Saini *et al*.[9].

2.4. Anti-elastase and anti-collagenase activity

Anti-elastase and anti-collagenase activities were determined according to methods of Kim *et al*.[17]. Anti-elastase assay involved incubation of fruit extracts with the porcine pancreatic elastase (stock solution 3.33 mg/mL in sterile water) for 15 min before adding the substrate. The final reaction mixture (250 μL) contained 0.2 mmol/L Tris-HCl buffer (pH 8.0), substrate 0.8 mmol/L N-succinyl–Ala-Ala-Ala-p-nitroanilide, 1 μg/mL elastase and 25 μL fruit extracts. Absorbance values between 381 nm and 402 nm (following pre-screen scans) were measured continuously for 20 min after adding substrate. EGCG (250 μmol/L) was used as a positive control and anti-elastase activity was expressed as mg EGCG equivalents/100 g FW.

For anti-collagenase assay, fruit extracts were incubated with the collagenase (0.8 IU/mL) in 50 mmol/L tricine buffer (400 mmol/L NaCl and 10 mmol/L $CaCl_2$, pH 7.5) for 15 min before adding substrate to start the reaction. The final reaction mixture (150 μL) contained 50 mmol/L tricine buffer, 0.8 mmol/L synthetic substrate N-[3-(2-furyl)acryloyl]-leu-gly-pro-ala, 0.1 IU collagenase and various dilutions of fruit extracts. Absorbance at 335 nm was

Table 1

List of fruits with their Latin names, common names, collection time and collection sites in Uttarakhand, India.

Botanical name	Common name	Voucher specimen	Collection time (2012–2014)	Collection sites
P. crenulata (D. Don) Roem. (Rosaceae)	Himalayan firethorn	113609	Sep	Ramgarhtalla, Nainital
B. asiatica Roxb. ex DC. (Barberidaceae)	Asiatic berberry	113590	Jul	Agrakhal
F. subincisa Buch.-Ham. ex Sm. (Moraceae)	Chanchri	113591	May	Agrakhal
M. serrata Roxb. (Moraceae)	Himalayan mulberry	113610	May	Ramgarhtalla, Nainital
Z. nummularia (Burm.f.) Wight & Arn. (Rhamnaceae)	Wild jujube	113594	Jan	Srinagar
L. asiatica (L.) Risdale (Leeaceae)	Kumali	113612	Nov	Agrakhal
D. capitata (Wall.) Hutch. (Cornaceae)	Bhamora	114568	Nov	Ramgarhtalla, Nainital
Z. mauritiana Lam. (Rhamnaceae)	Jujube	114637	Jan	Lansdown, Kotdwar
P. cerasoides D.Don (Rosaceae)	Himalayan wild-cherry	113595	Apr	Agrakhal
A. latifolia (Roxb.) Planch. (Vitaceae)	Jungle grape-vine	114572	Sep	Ramgarhtalla, Nainital
V. jacquemontii R. Parker (Vitaceae)	Wild grapes	114571	Sep	Ramgarhtalla, Nainital
M. alba L. (Moraceae)	Himalayan mulberry	114569	May	Neelkanth
G. optiva J.R. Drumm. ex Burrett (Tiliaceae)	Bhimal	113596	Nov	Ramgarhtalla, Nainital

measured immediately after adding substrate and then continuously for 20 min by using microplate reader (Fluostar Optima, BMG Labtech, Germany). EGCG (250 μmol/L) was used as positive control and the anti-collagenase activity was expressed as mg EGCG equivalents/100 g FW.

2.5. Anti-tyrosinase activity

Anti-tyrosinase activity was evaluated with respect to the fruit extracts ability to inhibit mushroom tyrosinase[18]. Reaction mixture including phosphate buffers (20 μL, 0.1 mol/L, pH 6.8), 3-4-dihydroxy-L-phenylalanine (20 μL, 0.85 mmol/L) as the substrate, and diluted fruit (20 μL) extracts were incubated at 25 ˚C for 10 min. Afterwards, mushroom tyrosinase (20 μL, 1 000 IU/mL) was added to initiate the reaction and incubated for 25 min. The absorbance was measured at 492 nm. Kojic acid (0.5 mg/mL in water) was used as positive control and anti-tyrosinase activity was determined as mg kojic acid equivalents/100 g FW.

2.6. Anti-inflammatory activity

Anti-inflammatory activity was determined with respect to the ability of fruit extracts to stabilize the human red blood cell (HRBC) membrane and inhibit the protease activity *in vitro*. HRBC membrane stabilization activity was determined as method mentioned by Awe *et al*.[19], and expressed as g diclofenac equivalent HRBC membrane stabilization activity (DEHRBCMSA)/100 g FW. Proteinase inhibition activity was evaluated according to the protocol mentioned by Kumar *et al*. by using trypsin as proteinase[20]. The proteinase inhibition activity of the fruit extracts was calculated as g catechin equivalent proteinase inhibition activity (CEPIA)/100 g FW by using catechin (2–10 mg/mL) as standard.

2.7. Statistical analysis

To rule out any inconsistency, three independent extractions were performed and each extract was analyzed at least 3 times for each parameter. The results were expressed as mean of 3 independent experiments (n = 3) with calculation of SE. Statistical analysis of the data was performed by using Microsoft Excel and Prism 3 Pad Software (Microsoft, Redmond, WA, USA).

3. Results

3.1. TPC and TFC

TPCs of fruit extracts were determined by using gallic acid as standard (R^2 = 0.999). TPC varied from (58.83 ± 14.50) to (4 496.39 ± 318.00) mg GAE/100 g FW (Table 2). It was highest in *A. latifolia* and lowest in *F. subincisa*. *L. asiatica* and *G. optiva* also showed significantly higher TPC (P < 0.05). A reasonably good level of TPC was observed in *B. asiatica* and *M. alba*. The order of TPC was *A. latifolia* > *L. asiatica* > *G. optiva* > *B. asiatica* ≥ *M. alba* > *V. jaquemonti* ≥ *Z. mauritiana* > *D. capitata* ≥ *M. serrata* > *P. cerasoides* > *Z. nummularia* ≥ *P. crenulata* > *F. subincisa*. TFC were determined by using catechin as standard (R^2 = 0.997). The range varied from (108.00 ± 4.00) to (1 963.75 ± 134.20) mg CE/100 g FW (Table 2). *L. asiatica* showed highest TFC and *P. cerasoides* showed lowest TFC. *G. optiva*, *A. latifolia* and *B. asiatica* fruit extracts showed significantly higher TFC than others (P < 0.05). The order of TFC was *L. asiatica* > *G. optiva* > *A. latifolia* ≥ *B. asiatica* > *V. jaquemonti* > *M. alba* > *M. serrata* > *D. capitata* ≥ *P. crenulata* > *F. subincisa* ≥ *Z. mauritiana* ≥ *Z. nummularia* ≥ *P. cerasoides*. A significant correlation (R^2 = 0.878, P < 0.05) was obtained between TPC and TFC among the tested fruits excluding *A. latifolia*.

Table 2
TPC and TFC in the acetone extracts of wild edible fruits.

Fruit extracts	TPC (mg GAE/100 g FW)	TFC (mg CE/100 g FW)
P. crenulata	144.16 ± 3.50	303.75 ± 3.50
B. asiatica	648.44 ± 24.00	1 208.83 ± 45.30**
F. subincisa	58.83 ± 14.50	189.37 ± 8.00
M. serrata	335.67 ± 6.10	458.33 ± 15.10
Z. nummularia	179.25 ± 7.00	148.50 ± 1.50
L. asiatica	1 860.49 ± 64.80*	1 963.75 ± 134.20
D. capitata	356.66 ± 12.00	337.75 ± 19.50
Z. mauritiana	520.00 ± 17.50	179.99 ± 7.50
P. cerasoides	239.63 ± 22.50	108.00 ± 4.00
A. latifolia	4 496.39 ± 318.00	1 280.00 ± 40.00**
V. jacquemontii	551.42 ± 29.00	800.00 ± 0.00
M. alba	622.85 ± 72.50	669.99 ± 20.50
G. optiva	1 425.00 ± 28.00*	1 580.00 ± 15.00**

Each value was expressed as mean ± SE (n = 3). *: Significantly higher TPC (P < 0.05) than the rest; **: Significantly higher TFC (P < 0.05) than others.

3.2. Antioxidant activity

DPPH radical scavenging activity was determined by using ascorbic acid as standard (R^2 = 0.997). Observations showed presence of potent DPPH radical scavenging activity in all fruit extracts (Table 3). It ranged from (101.96 ± 2.50) to (7 746.71 ± 0.00) mg ascorbic acid equivalents/100 g FW in studied fruits, being highest in *A. latifolia* and lowest in *P. cerasoides* fruit extracts. The DPPH radical scavenging activities of *L. asiatica* and *B. asiatica* were also significantly higher than remaining fruits (P < 0.05).

$ABTS^+$ radical scavenging activity was determined using butylated hydroxyanisole as a standard (R^2 = 0.987). $ABTS^+$ radical scavenging activity was highest in *A. latifolia* and lowest in *F. subincisa*. *L. asiatica* fruit extracts showed significantly higher (P < 0.05) $ABTS^+$ radical scavenging activity than that of *B. asiatica*, *V. jaquemonti* and rest of the fruits (Table 3). Superoxide radicals scavenging activity was determined using catechin as standard (R^2 = 0.993). It was the highest in *L. asiatica* and the lowest in *F. subincisa* (Table 3). *A. latifolia* and *G. optiva* showed significantly higher (P < 0.05) superoxide radicals scavenging activity than rest of the fruits. The linoleate hydroperoxide radical scavenging activity was measured by using butylated hydroxyanisole as a standard (R^2 = 0.912). The highest and lowest linoleate hydroperoxide radical scavenging activity were found in *L. asiatica* and *F. subincisa,* respectively (Table 3). *A. latifolia* and *M. serrata* fruit extracts also showed significantly greater (P < 0.05) linoleate hydroperoxide radical scavenging activity than other fruit extracts. Ferric reducing activity was determined by using ascorbic acid as a standard (R^2 = 0.912). It was detected at highest level in *A. latifolia* extracts followed by *L. asiatica*, *B. asiatica* and *D. capitata* fruit extracts (Table 3). The ferric reducing activity of these fruits was significantly higher than that of other fruit extracts (P < 0.05). The *F. subincisa* fruit extracts showed lowest ferric reducing activity. The ferrous metal chelating activity of fruit extracts was evaluated on the basis of their ability to chelate ferric ions by using ethylene diamine tetraacetic acid as standard (R^2 = 0.982). It was found to be the highest in *A. latifolia* and the lowest in *P. crenulata* (Table 3). *G. optiva* and *M. serrata* did not show significant difference in ferrous metal chelating activity compared to *A. latifolia* (P > 0.05); however, it was found to be significantly different from that of other fruit extracts (P < 0.05).

In order to establish the influence of TPC and TFC on individual antioxidant activity, correlation studies were carried out. The observation revealed differential correlation coefficient for TPC and TFC with the individual antioxidant activity of the fruit extracts (Table 4). The DPPH radical scavenging activity, $ABTS^+$ radical

Table 3
Antioxidant activities of wild edible fruits.

Fruit extracts	DPPHRSA	ABTSRSA	SORSA	LPRSA	FRA	FMCA
P. crenulata	657.36 ± 14.00	251.50 ± 23.00	4 247.95 ± 862.40	2 774.54 ± 23.90	275.00 ± 50.00	9.66 ± 1.00
B. asiatica	1 579.10 ± 28.10[a]	770.48 ± 5.10	7 443.94 ± 1 260.00	3 941.32 ± 24.44	1 678.30 ± 40.50[e]	23.45 ± 0.00
F. subincisa	292.31 ± 9.50	84.70 ± 0.00	1 057.50 ± 169.00	1 089.89 ± 20.40	65.50 ± 1.50	29.07 ± 3.50
M. serrata	817.90 ± 147.70	437.18 ± 8.60	3 683.44 ± 1 026.00	5 363.31 ± 42.71[d]	751.67 ± 34.20	43.18 ± 0.33[f]
Z. nummularia	286.53 ± 19.00	230.18 ± 5.50	6 550.03 ± 468.50	2 325.36 ± 18.00	284.00 ± 18.00	19.84 ± 6.99
L. asiatica	3 990.10 ± 18.10[a]	2 989.00 ± 154.50[b]	52 500.71 ± 1 345.00	10 019.26 ± 395.30	6 366.60 ± 409.00[e]	15.37 ± 4.80
D. capitata	952.68 ± 9.50	645.93 ± 7.50	7 152.35 ± 5.00	2 773.76 ± 454.50	1 262.50 ± 42.50[e]	13.23 ± 2.50
Z. mauritiana	204.57 ± 5.00	220.00 ± 16.50	1 843.46 ± 18.00	2 111.73 ± 90.00	289.50 ± 17.50	17.37 ± 2.00
P. cerasoides	101.96 ± 2.50	195.03 ± 8.50	2 577.22 ± 18.50	2 139.84 ± 36.50	167.00 ± 21.00	30.15 ± 4.50
A. latifolia	7 746.71 ± 0.00	4 057.55 ± 51.00	32 650.85 ± 164.00[c]	6 511.41 ± 0.00[d]	8 250.00 ± 50.00[e]	49.41 ± 0.00[f]
V. jacquemontii	577.61 ± 43.00	765.41 ± 7.00	6 365.22 ± 67.00	2 948.63 ± 0.00	225.00 ± 35.00	17.60 ± 0.00
M. alba	420.09 ± 6.50	394.74 ± 0.50	3 494.24 ± 144.00	3 996.25 ± 54.00	490.00 ± 5.00	32.52 ± 1.00
G. optiva	766.13 ± 0.50	664.45 ± 16.00	11 787.87 ± 36.40[c]	2 645.30 ± 779.00	1 055.00 ± 5.00	48.57 ± 1.00[f]

DPPHRSA: DPPH radical scavenging activity; ABTSRSA: $ABTS^+$ radical scavenging activity; SORSA: Superoxide radicals scavenging activity; LPRSA: Linoleate hydroperoxide radical scavenging activity; FRA: Ferric reducing activity; FMCA: Ferrous metal chelating activity. ABTSRSA and LPRSA were expressed as mg butylated hydroxyanisole equivalent/100 g FW; DPPHRSA and FRA were expressed as mg ascorbic acid equivalent/100 g FW; SORSA and FMCA were expressed as mg CE and mg EDTA equivalents/100 g FW, respectively. Each value was expressed as mean ± SE (n = 3). [a]: Significantly higher DPPHRSA than the remaining fruits ($P < 0.05$); [b]: Significantly higher ABTSRSA than the rest of other fruits ($P < 0.05$); [c]: Significantly higher SORSA than rest of the fruits ($P < 0.05$); [d]: Significantly higher LPRSA than that of other fruits extracts ($P < 0.05$); [e]: Significantly higher FRA than that of other fruit extracts ($P < 0.05$); [f]: Significantly different FMCA than that of other fruit extracts ($P < 0.05$).

scavenging activity and ferric reducing activity showed strong correlation with TPC of fruit extract while showing no significant correlation with TFC. In contrast, superoxide radicals scavenging activity and linoleate hydroperoxide radical scavenging activity established better correlation with TFC than TPC of fruit extract. Conversely, ferrous metal chelating activity showed no significant correlation to either of TPC or TFC.

Table 4
Pearson correlation coefficients between antioxidant activities and TPC/TFC (at significance $P < 0.05$).

Assays	Correlation coefficients (R^2)	
	TPC	TFC
DPPHRSA	0.909*	0.511
ABTSRSA	0.869*	0.491
SORSA	0.515	0.625
LPRSA	0.368	0.541
FRA	0.839*	0.480
FMCA	0.267	0.083

DPPHRSA: DPPH radical scavenging activity; ABTSRSA: $ABTS^+$ radical scavenging activity; SORSA: Superoxide radicals scavenging activity; LPRSA: Linoleate hydroperoxide radical scavenging activity; FRA: Ferric reducing activity; FMCA: Ferrous metal chelating activity. *: Strong correlation of DPPHRSA, ABTSRSA and FRA showed with TPC of fruit extract.

3.3. Anti-elastase and anti-collagenase activity

Elastase and collagenase are located in extracellular matrix responsible for breaking elastin and collagen fiber, the main components of extracellular matrix and thus leading to loosening of skin resulting in skin aging. Anti-elastase and anti-collagenase activities of the fruit extracts were determined by using EGCG as standard inhibitor (R^2 = 0.991 and R^2 = 0.963, respectively). The observations showed the highest and the lowest anti-elastase activities in *B. asiatica* and *M. alba* fruit extracts, respectively (Table 5). A significantly higher level of anti-elastase activity was also observed in *P. cerasoides*, *P. crenulata*, *L. asiatica*, *A. latifolia* and *G. optiva* than in other fruit extracts. Anti-collagenase activity of the fruit extracts was found to be highest in *L. asiatica* ($P < 0.05$) and lowest in *Z. nummularia* (Table 5). *P. crenulata* showed the second highest anti-collagenase activity while *M. serrata* and *P. cerasoides* fruit extracts showed fair levels of it, significantly higher than those of rest of the fruits extracts ($P < 0.05$). In addition, anti-elastase and anti-collagenase activities were found to be neither correlated with phenolics nor with flavonoids.

It was significant to note that *B. asiatica* and *G. optiva* fruit extracts showed excellent anti-elastase activities while exhibiting comparatively lower anti-collagenase activities. In contrary, *M.*

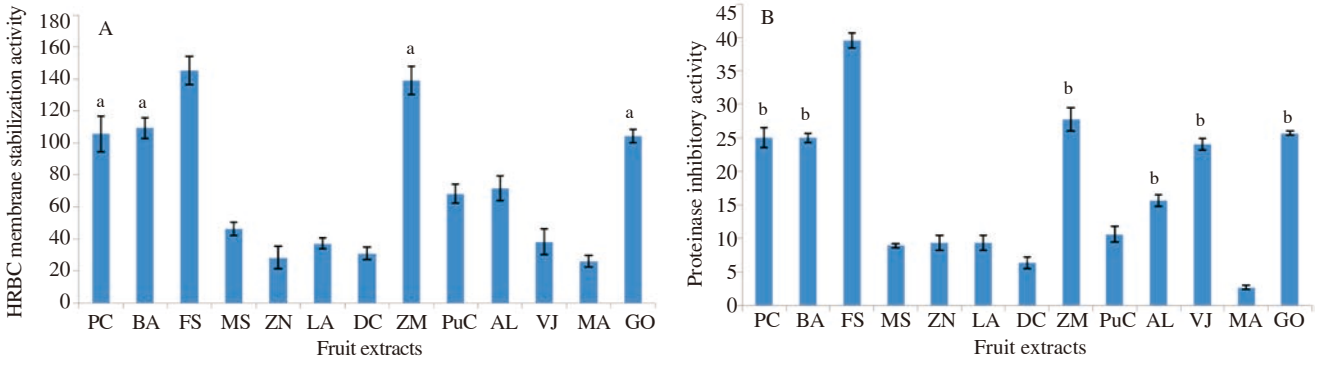

Figure 1. *In vitro* anti-inflammatory activities of wild edible fruit extracts.
A: HRBC membrane stabilization activity was represented as mg diclofenac equivalent HRBC membrane stabilization activity/100 g FW; B: Proteinase inhibition activity of wild edible fruits was represented as mg CEPIA/100 g FW; Each value was represented mean ± SE (n = 3). PC: *P. crenulata*; BA: *B. asiatica*; FS: *F. subincisa*; MS: *M. serrata*; ZN: *Z. nummularia*; LA: *L. asiatica*; DC: *D. capitata*; ZM: *Z. mauritiana*; PuC: *P. cerasoides*; AL: *A. latifolia*; VJ: *V. jacquemontii*; MA: *M. alba*; GO: *G. optiva*. [a]: Significantly higher HRBC membrane stabilization activity than others ($P < 0.05$); [b]: Significantly higher proteinase inhibition activity than that of remaining fruits ($P < 0.05$).

serrata and *V. jaquemonti* fruit extracts demonstrated noteworthy anti-collagenase activities while displaying relatively lower anti-elastase activities. However, *L. asiatica*, *P. crenulata P. cerasoides* and *A. latifolia* fruit extracts exhibited remarkable inhibitory activities against both elastase and collagenase enzymes.

Table 5

Anti-elastase, anti-collagenase and anti-tyrosinase activities of fruit extracts.

Fruit extracts	Anti-elastase	Anti-collagenase	Anti-tyrosinase
P. crenulata	1 551.30 ± 26.11[a]	4 377.53 ± 54.74[b]	23 029.68 ± 398.00[c]
B. asiatica	1 751.78 ± 20.67	1 767.40 ± 188.58	65 162.86 ± 228.00
F. subincisa	297.23 ± 4.19	651.77 ± 39.18	1 762.63 ± 23.00
M. serrata	411.51 ± 10.38	3 914.52 ± 3.88[b]	1 465.45 ± 37.00
Z. nummularia	411.02 ± 9.50	622.29 ± 38.92	762.09 ± 16.02
L. asiatica	1 426.72 ± 5.21[a]	8 560.90 ± 77.42	30 661.60 ± 1849.00[c]
D. capitata	109.43 ± 3.73	2 189.19 ± 36.18	7 246.40 ± 296.00
Z. mauritiana	260.79 ± 8.94	772.06 ± 0.00	1 012.88 ± 9.70
P. cerasoides	1 568.34 ± 20.19[a]	3 753.15 ± 71.09[b]	17 417.66 ± 375.30
A. latifolia	1 176.46 ± 22.05[a]	2 382.11 ± 49.90	12 752.80 ± 282.00
V. jacquemontii	291.79 ± 10.90	3 180.68 ± 32.00	24 625.04 ± 619.00[c]
M. alba	122.47 ± 8.75	1 741.34 ± 56.31	14 471.95 ± 274.00
G. optiva	1 043.71 ± 43.00[a]	1 622.93 ± 22.93	13 556.70 ± 311.00

Anti-elastase activities and anti-collagenase activities were represented as mg ECGC equivalents/100 g FW; Anti-tyrosinase activities of wild edible fruits were represented as mg kojic acid equivalents/100 g FW; Each value was represented by mean ± SE (n = 3). [a]: Significantly higher anti-elastase activity than rest of the fruits extracts ($P < 0.05$); [b]: Higher anti-collagenase activity than rest of the fruits extracts ($P < 0.05$); [c]: Significantly higher levels of anti-tyrosinase activities ($P < 0.05$) than rest of the fruit extracts.

3.4. Anti-tyrosinase activity

Anti-tyrosinase activity of the fruit phenolics was determined by using kojic acid as standard inhibitor ($R^2 = 0.969$) of melanin biosynthetic pathway. It ranged from (762.09 ± 16.02) to (65 162.86 ± 228.00) mg kojic acid equivalents/100 g FW (Table 5). It was found to be highest in *B. asiatica* while lowest in *Z. nummularia*. Although, all fruit extracts showed substantial levels of anti-tyrosinase activities, *L. asiatica*, *V. jaquemonti* and *P. crenulata* exhibited significantly higher levels of anti-tyrosinase activities ($P < 0.05$). No correlation was observed between anti-tyrosinase activity and TPC/TFC of fruit extracts.

3.5. Anti-inflammatory activity

Anti-inflammatory activities of the fruit extracts were studied with respect to their ability to stabilize HRBC membrane (HRBC membrane stabilization activity) and proteinase inhibition activity *in vitro*. The study demonstrated presence of HRBC membrane stabilization activity in all the 13 wild edible fruit extracts as determination by using standard curve of diclofenac ($R^2 = 0.965$), a known anti-inflammatory drug (Figure 1A). Maximum HRBC membrane stabilization activity was detected in *F. subincisa* (145.30 ± 8.60) mg diclofenac equivalent HRBC membrane stabilization activity/100 g FW), while minimum was observed in *M. alba* fruit extracts (26.09 ± 3.70) mg diclofenac equivalent HRBC membrane stabilization activity/100 g FW). Among other fruit extracts, *Z. mauritiana*, *B. asiatica*, *P. crenulata* and *G. optiva* showed significantly higher HRBC membrane stabilization activity than others ($P < 0.05$). The order of HRBC membrane stabilization activity of fruit extracts was found to be *F. subincisa* > *Z. mauritiana* ⩾ *B. asiatica* ⩾ *P. crenulata* ⩾ *G. optiva* ⩾ *A. latifolia* > *P. cerasoides* > *M. serrata* > *V. jacquemontii* > *L. asiatica* > *D. capitata* > *Z. nummularia* > *M. alba*.

Proteinase inhibition activity of fruit phenolics was studied by using catechin as standard proteinase inhibitor ($R^2 = 0.854$) with trypsin as proteinase (Figure 1B). The study revealed maximum proteinase inhibition activity in *F. subincisa* (39.53 ± 1.15) CEPIA/100 g FW and minimum in *M. alba* (2.716 ± 0.33) CEPIA/100 g FW). *Z. mauritiana*, *G. optiva*, *P. crenulata*, *B. asiatica*, *V. jacquemontii* and *A. latifolia* fruit extracts did not show any significant difference in their proteinase inhibition activity ($P > 0.05$), although they did show significantly higher proteinase inhibition activity than that of remaining fruits ($P < 0.05$).The order of proteinase inhibition activity was *F. subincisa* > *Z. mauritiana* ⩾ *G. optiva* ⩾ *P. crenulata* ⩾ *B. asiatica* ⩾ *V. jacquemontii* > *A. latifolia* > *P. cerasoides* ⩾ *Z. nummularia* ⩾ *L. asiatica* ⩾ *M. serrata* ⩾ *D. capitata* > *M. alba*. The HRBC membrane stabilization activity and proteinase inhibition activity of fruit extracts did not show any correlation with phenolic or flavonoid contents.

4. Discussion

In the present study, the fruit extracts were prepared in 80% acetone as it was considered previously as better solvent over others for extraction of polyphenols[6-7,11]. Our study is the first one to report polyphenolic contents of *A. latifolia*, *L. asiatica*, *G. optiva*, *D. capitata*, *F. subincisa*, *P. cerasoides* and *V. jacquemontii* fruits. However, there are few studies showing notable polyphenol contents in *P. crenulata*[14,21], *B. asiatica*[21], *M. alba*[22-24], *Z. nummularia* and *Z. mauritiana* fruit extracts with varied levels[6,25]. A significant correlation between TPC and TFC was in accordance with earlier studies which suggested that fruits with high phenolics might likely have higher flavonoid contents[6].

Antioxidant activities have not been studied in most of the selected wild edible fruits except few such as *P. crenulata*, *B. asiatica*, *Z. mauritiana*, *Z. nummularia* and *M. alba*[6,14,21,23-25]. *P. crenulata* fruit extract was shown to possess fair levels of ABTS radical scavenging activity, DPPH radical scavenging activity and ferric reducing activity[14], although, lesser than that of *B. asiatica* fruit extracts[21]. *M. alba* fruit extracts were previously reported to own significant DPPH radical scavenging activity and linoleate hydroperoxide radical scavenging activity[23,24]. Few studies on *Z. nummularia* and *Z. mauritiana* fruit extracts demonstrated presence of significant DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing activity and H_2O_2 radical scavenging activities[6,25]. The findings indicating differential contribution of TPC and TFC in various antioxidant activities are quite surprising and contrary to the earlier studies which showed strong correlation of both TPC and TFC with DPPH radical scavenging activity, ABTS radical scavenging activity and ferric reducing activity[3,6]. However, present study employed aluminum chloride method for evaluation of TFC which indeed predominantly detected flavones and flavonols[16]. It was possible that the difference in our finding might be attributed to the presence of some other classes of flavonoids other than flavones and flavonols in studied fruits which remained under-detected by the employed method.

Our observations of differential anti-elastase and anti-collagenase activities of fruit extracts were in agreement with previous report that showed differential anti-collagenase and anti-elastase activities of extracts from 21 plant species[26]. Anti-elastase, anti-collagenase and anti-tyrosinase activities of polyphenols have been widely reported[17,27-29]. However, out of 13 studied fruits, only the leaves and root of *M. alba* had been previously studied as potent source of anti-tyrosinase activities[29].

Anti-inflammatory activities of some of the fruits under study namely *A. latifolia* and *P. crenulata* have recently been studied. Das *et al*. revealed presence of dose dependent anti-inflammatory activities in the ethanolic fruit extracts of *A. latifolia* (Roxb.) by using carrageenan-induced rat paw edema method at the doses of 250 mg/kg and 500 mg/kg[30], while the research of Bahuguna and Rawat showed significant HRBC membrane stabilization activity and proteinase inhibition activity in *P. crenulata* fruit extracts[31].

Present study is the first meticulous investigation on evaluation and comparison of polyphenol contents, antioxidant, anti-elastase, anti-collagenase and anti-tyrosinase activities in 13 wild edible fruits of North-West Himalayan Region of India which demonstrates their higher polyphenol contents, high efficiency in scavenging harmful free radicals and reducing ferric ions, higher effectiveness in inhibiting enzymes of skin aging and melanin biosynthetic pathway. The study also revealed differential contribution of TPC and TFC in various free radical scavenging and ferric reducing activities of the fruit extracts. Conversely, no evident relationship was observable between the anti-elastase/anti-collagenase/anti-tyrosinase/anti-inflammatory activities and TPC/TFC of the fruit extracts implying role of individual or combination of specific phenolics and flavonoids in aforesaid activities of wild edible fruit extracts. The capability of Himalayan wild edible fruits to scavenge diverse free radicals, inhibit enzymes responsible for skin aging, skin darkening are highly commendable suggesting their dietary intake to maintain the antioxidant levels of body which may in turn prevent the free radicals mediated pathological diseases together with skin aging and skin darkening. In addition, fruit extracts could also be employed to develop safe and effective formulation for general health maintenance and effective anti-skin aging, skin whitening cosmetics.

Conflict of interest statement

We declare that we have no conflict of interest.

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