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Phytochemicals, mineral contents and antioxidant property of wild edible fruits of Sikkim Himalaya

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This study aimed to investigate four wild edible fruits of Sikkim Himalaya viz., *Baccaurea sapida, Diploknema butyracea, Machilus edulis* and *Spondias axillaris* for their mineral content, phytochemicals (ascorbic acid, β-carotene, flavonoids, lycopene and total phenolic contents) and antioxidant property. The total phenolic and lycopene contents were found maximum in *S. axillaris* subsequently in *M. edulis*, *D. butyraceae* and *B. sapida*. *S. axillaris* also showed the highest antioxidant activities and contains significantly higher concentrations of calcium, phosphorus, potassium, sodium, magnesium and iron. The amount of ascorbic acid was observed highest in *D. butyracea*, followed by *S. axillaris, B. sapida* and *M. edulis,* whereas β-carotene was found to be highest in *M. edulis*. Results reinforce the promotion of these underutilized wild edible fruits, especially, *S. axillaris* and *M. edulis* as natural sources of phytochemicals, minerals and antioxidant compounds for food and pharmaceutical industries.

Keywords: Antioxidant capacity, β-Carotene, Himalaya, Mineral content, Wild edible fruits

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The Sikkim Himalayan region is enriched with enormous phytodiversity due to its diverse eco-geographical and eco-climatic conditions. From this region, a total of 190 plant species have been reported as wild edibles which are consumed frequently by the local inhabitants as supplementary food[1]. These wild edibles have a crucial role in food security of the rural populations because they are available in seasons when conventional staple crops, vegetables and fruits are not available. The plethora of wild edibles present in the region is not only the sources of food, nutritionally balanced diet and medicine but also provide livelihood options to marginalized families. According to Sundriyal *et al.*[2] the annual turnover of wild edibles in rural markets of Sikkim state is approximately 140 tons, with the gross earnings of 0.022 million dollors. It clearly indicates that the wild edible species of the region contribute significantly to the earnings of the underprivileged families. It is likely that the increased production potential, sustainable harvests of useful parts and validation of purported nutritional value of different wild edible plants can boost the local economy.

Wild edible fruits are considered as healthy dietary supplements and consumed by human beings since time immemorial as a natural source of nutraceutical compounds[3]. However, despite the profound economic and ecological value of wild edible species of Sikkim Himalayan region, very little scientific work is done in wild edible fruits. Although, some reports on wild edible fruits are available in other parts of the Indian Himalayan region such as *Berberis asiatica*[4,5], *Ficus palmata*[6], *Morus alba*[7,8], *Myrica esculenta*[5,9], *Phyllanthus emblica*[5,10], *Pyracantha crenulata*[5,7]. However, limited biochemical studies on wild edible fruits of Sikkim Himalaya are available so far and majority of these reports deal with documentation, field investigation and preliminary work on nutritive value[1,11-14]. Since the research on wild edible fruits of Sikkim Himalaya is scant, thus, it is crucial to perform in-depth research on these fruits. This study was aimed to investigate (i)

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Abbreviations: Abs: absorbance; ABTS: 2, 2-azinobis-3-ethylbenzothiazoline-6 sulfonic acid; BHT: Butylated hydroxytoluene; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FC: Folin ciocalteu; GAE: Gallic acid equivalent; ICP-MS: Inductively Coupled Plasma Mass Spectrophotometry; TPC: Total Phenolic content; TFC: Total flavonoid content; QE: Quercetin equivalent

phytochemicals (total phenolic, flavonoid, lycopene, β-carotene and ascorbic acid contents), (ii) mineral composition and (iii) antioxidant properties (ABTS and DPPH) of selected wild edible fruits of Sikkim Himalaya. The taxonomic details and market values of selected wild edible fruits used for the present investigation are listed in Table 1.

Materials and Methods

Fruits collection

Fresh, adequately matured and healthy fruits of selected wild edible species (*Baccaurea sapida, Diploknema butyracea, Machilus edulis* and *Spondias axillaris*) were collected in three lots (one from Gangtok, Sikkim market and two from the natural populations) (Fig. 1 & Table 1). All the collected fruits were brought to the laboratory for further experimentation at G.B. Pant National Institute of Himalayan Environment (NIHE), Sikkim Regional Centre, Gangtok. Of the collected lots, 2 kg fruits of uniform size and colour of each species were selected and pooled (random sampling) for further experimentation.

Chemicals and reagents

Analytical grade chemical and reagents were used in the present investigation. Amongst, ascorbic acid, gallic acid and quercetin were procured from Sigma–Aldrich (Steinheim, Germany); 2,2-Azinobis-3-

Fig. 1 — Pictorial presentation of wild edible fruits of Sikkim Himalaya; (A) *B. sapida*; (B) *D. butyracea*; (C) *M. edulis*; (D) *S. axillaris.*

Table 1 — Details of selected wild edible fruits of the Sikkim Himalaya.

Wild Edible fruits [common name]	Family	Distribution range	Local use	Sampling location	Collection time	Local market price INR
Spondias axillaris Roxb., [Lupsee]	Anacardiaceae	Throughout the Himalaya between 300-1500m asl	Consumed raw and is also a good source of pickle which has a long shelf-life	1. Temi Tarku, South Sikkim (27° 14' 23.28"N; 88° 25' 39.51"E) 2. Kalimpong, West Bengal (27° 03' 41.04''; 88° 27' 46.05'')	July-February	30-85/kg.
Machilus edulis King [Pumsee]	Lauraceae	Nepal, Bhutan, India (Sikkim, Arunachal Pradesh and the whole north eastern region) in the temperate forests across 1700 m asl	Ripened fruit pulp is considered as a good supplement for weight management	1. Temi Tarku, South Sikkim (27° 14' 23.28"N; 88° 25' 39.51"E) 2. Kalimpong, West Bengal (27° 03' 41.04''; 88° 27' 46.05'')	November to February	100-200/kg
Diploknema butyracea (Roxb.) Lam [Chiuree]	Sapotaceae	Throughout the Himalaya between 300-1300 m asl	Seeds are used to produce butter that is used in making medicine, soaps and candles	Temi Tarku, South Sikkim (27° 14' 23.28"N; 88° 25' 39.51"E) Kamrang, South Sikkim (27° 09' 93.01"N; 88° 21' 28.80"E)	May-July	70-80 /kg
Baccaurea sapida (Roxb.) Muell.-Arg [Kusum]	Euphorbiaceae	Across the sub-Himalayan tract	The yellowish ripened fruit can be eaten as raw or are seldom used for making chutney	Temi Tarku, South Sikkim (27° 14' 23.28"N; 88° 25' 39.51"E) Namchi, South Sikkim (27° 09' 52.78"N; 88° 21' 59.49"E)	July-September	70-90 /kg

ethylbenzthiazoline-6-sulphonic acid (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), lycopene and β-carotene were bought from HiMedia, Pvt. Ltd. (Mumbai, India).

Extraction

Fruits of selected species were cleaned properly using tap water and followed by rinsing with double distilled water. Thereafter, peel and seeds of fruits were separated and fruit pulp was dried in an oven at 40±2 ºC. The fruit pulp was then crushed using grinder into fine powder and stored till further experimentations. The powdered material (2 g) of each fruit was extracted with 10 mL ethanol solution for 24 h and extract was filtered using Whatman filter paper no.1. The liquid phase (filtrate) was then concentrated by drying at 40° C in an oven. This ethanolic extract was used to evaluate the antioxidant properties and quantification of phenolics and flavonoids. One portion of the fresh fruit samples, stored at 4 °C, extracted with methanol and processed for lycopene, *β*-carotene and ascorbic acid estimation.

Sample analysis

Mineral analysis

A total of nine minerals viz., calcium, copper, iron, manganese, magnesium, potassium, phosphorus, sodium and zinc were analyzed in the pulp of selected wild edible fruits. Dried fruit pulp (0.5 g) was subjected to microwave assisted digestion (Anton par microwave 3000) with 9 mL of 69% nitric acid and 2 mL hydrochloric acid. Thereafter, analysis of ionic constitution was performed using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS; Perkin Elmer Nex ION 300X.). Each element value was expressed as mg/100 g dw.

Determination of lycopene and β-carotene

β-Carotene and lycopene content was estimated by following Nagata & Yamashita[15]. The procedure involved, transfer of 100 mg of fruit methanolic extract into a test tube having 10 mL of acetone–hexane mixture (2:3). After 1 min of vigorous shaking at ambient temperature, the absorbance was read at 453, 505, 645 and 663 nm using UV-spectrophotometer (UV-1800, Shimadzu, Japan). Quantification of β-carotene and lycopene was done using following equations:

Lycopene (mg/ 100 mL) = - 0.0458 $Absorbance_{663}$+0.372 $Absorbance_{505}$-0.0806 $Absorbance_{453}$;

β-carotene (mg/ 100 mL)=0.216 $Absorbance_{663}$-0.304 $Absorbance_{505}$+0.452 $Absorbance_{453}$.

The data were expressed as µg /100 g of extract.

Determination of ascorbic acid

To quantify ascorbic acid content in fruit extracts, the procedure of Ferreira *et al*.[16] was used. Briefly, 100 mg of dried methanolic extract was dissolved in 10 mL of extracting solution containing 5% metaphosphoric acid in 10% acetic acid. The mixture was kept for 45 min at room temperature thereafter filtered using Whatman filter paper No. 4. Subsequently, 1.0 mL of filtrate was added to the 9 mL of 2, 6-dichlorophenolindophenol (0.005%) and absorbance of the resultant solution was measured after 30 min at 515 nm using the UV spectrophotometer. The ascorbic acid standard curve (0.01–0.10 mg/mL) was used for the quantification and the results were expressed as mg of ascorbic acid/100 g of extract.

Determination of TPC

The total phenolic content (TPC) was assessed by following Meda *et al.*[17]. In brief, 100 µL of 5 mg/mL fruit extract was mixed in 2.5 mL of Folin Ciocalteu reagent (diluted 10 times) and after 3 min, 2.0 mL sodium carbonate solution (7.5%) was added to it. The absorbance of the resultant mixture was taken after 30 min of incubation at 760 nm. Gallic acid standard curve was used for quantification and TPC of extracts was measured as milligrams gallic acid equivalents (mg GAE/g extract).

Determination of TFC

Total flavonoid content (TFC) was determined using aluminum chloride method, with minor modifications[18]. In brief, individual dry extracts were dissolved in methanol initially and 1 mL of each extract solution was mixed with equal volume of 2% aluminum chloride methanolic solution and left for 10 min incubation at room temperature. The absorbance of the resultant color was read at 410 nm using the UV spectrophotometer. The quercetin standard curve was used for quantification and TFC was estimated as milligram quercetin equivalent (mg QE/g extract).

Determination 2, 2-diphenyl-1-picrylhydrazyl (DPPH) activity

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of extracts was determined by the method originally developed by Sarikurku[19]. Appropriate dilutions of the extracts solution (1 mL)

were mixed with 4 mL of 0.004% DPPH methanolic solution. After 30 min, the absorbance of the resultant mixture was read at 517 nm under UV-spectrophotometer. The butylated hydroxytoluene (BHT) was used as the standard. The following equation was used for the quantification of DPPH radical scavenging potential of samples:

DPPH radical scavenging activity (%)=[(Absorbance of control–Absorbance of sample)]/[(Absorbance of control) x 100.

Where, DPPH solution+ethanol is control; DPPH solution+extract/BHT is sample. The radical scavenging ability of samples was determined by IC_{50} values. IC_{50} value of a sample indicates the sample concentration at which 50% of DPPH radicals were scavenged.

Determination of 2, 2-azinobis-3-ethylbenzothiazoline-6 sulfonic acid (ABTS) activity

The ABTS (2, 2-azinobis-3-ethylbenzothiazoline-6 sulfonic acid) radical scavenging activity of extracts was estimated by following Re *et al.*[20]. Briefly, for preparing working solution, an equal amount of ABTS (7 mM) and ammonium persulfate was mixed and diluted with methanol until 0.706±0.02 absorbance was obtained at 734 nm. Thereafter, 1.5 mL of fruits extracts were mixed with the equal quantity of working ABTS solution and kept in dark, after 7 min the absorbance was measured at 734 nm using the UV spectrophotometer. The BHT was used as the standard to compare ABTS scavenging capacity.

ABTS radical scavenging activity (%)= [(Absorbance of control – Absorbance of sample)]/ [(Absorbance of control)] x 100. Where ABTS working solution+ ethanol is control; ABTS working solution+extract/standard is sample. IC_{50} value was calculated to determine the ABTS radical scavenging activity of extracts as mentioned above in the DPPH assay.

Statistical analysis

All analytical experiments performed in present investigation were repeated thrice and results are expressed as mean±standard deviation (SD). The data analysis was done using SPSS software version 17 and Duncan's multiple range test (DMRT) was used to separate mean values of each parameter at $p \leq 0.05$ significant levels.

Results and Discussion

Minerals content

Minerals are involved in several biochemical processes which are vital for human body to maintain normal growth and development[21]. Insufficient intake of minerals leads to several nutritional deficiency diseases such as improper physical and intellectual development and weak immune system. Therefore, it is obligatory to investigate mineral contents in edible fruits and plants to identify mineral-rich foods.

In the present study, four wild edible fruits of Sikkim Himalaya have been investigated for five macro-minerals (calcium, magnesium, potassium, phosphorus, and sodium) and four micro-minerals (manganese, copper, iron and zinc). Table 2 describes the macro and micro-mineral level in the studied wild edible fruits. Amount of macro-minerals namely calcium, magnesium, potassium, phosphorus and sodium in studied fruits varied from 1.92 to 13.90 mg/100 g, 24.93 to 74.44 mg/100 g, 301.35 to 711.43 mg/100 g, 52.89 to 78.05 mg/100 g and 0.68 to 1.73 mg/100 g, respectively. Among the investigated fruits, *S. axillaris* contained the highest amount of calcium (13.90 mg/100 g), magnesium (74.44 mg/100 g), potassium (711.43 mg/100 g), sodium (1.73 mg/100 g) and phosphorus (78.05 mg/100 g). *M. edulis* contained the second highest amount of potassium (464.36 mg/100 g), magnesium (35.71 mg/100 g) and phosphorus (70.86 mg/100 g). Contents of micro-minerals viz. manganese, iron, copper, and zinc varied from 0.16 to 8.57 mg/100 g, 3.64 to 25.17 mg/100 g, 1.32 to 5.77 mg/100 g and 0.08 to 0.41 mg/100 g, respectively. Manganese, copper and zinc were found to be highest in *M. edulis*, whereas the highest iron content was detected in *S. axillaris* (Table 2). The most abundant mineral in the investigated wild edible fruits of Sikkim was potassium which is in accordance with the report of Özcan *et al.*[22]. Thus,

Table 2 — Mineral composition of the wild edible fruits consumed in Sikkim Himalaya (mg/100 g)

Wild Edible fruits	Ca	K	P	Na	Mg	Mn	Fe	Cu	Zn
Baccaurea sapida	2.98	301.35	67.15	1.31	30.72	4.32	10.07	2.72	0.16
Diploknema butyraceae	2.89	410.42	52.89	0.68	24.93	0.16	3.64	1.32	0.08
Machilus edulis	1.92	464.36	70.86	1.22	35.71	8.57	5.63	5.77	0.41
Spondias axillaris	13.90	711.43	78.05	1.73	74.44	8.29	25.17	1.63	0.16

mineral analysis results indicate that the investigated wild edible fruits contain a relatively comparable amount of minerals than the common and widely consumed fruits and to some extent, can meet the daily requirement of these minerals.

Extraction yield, total phenolic content and total flavonoid content

In this study, ethanol was used for extraction as in our previous study it was identified as the most effective solvent for extraction of total phenols and total flavonoids in wild edible fruits of Sikkim Himalaya (unpublished data). Extraction yields varied from 8.32% to 19.85% for fruit extracts and results are given in Table 3. The fruit extracts yields were obtained in the following order: *M. edulis*> *S. axillaris*>*B. Sapida*>*D. butyraceae*.

Fruits contain ample amount of phenolic compounds that impart numerous health benefits besides nutrition[23]. This ubiquitous group of plant secondary metabolite is considered as most important compounds which content in fruits is strongly dependent on the various factors such as climate, edaphic factors, geographic locality, degree of ripeness, variety and storage conditions[24].

Among the evaluated fruit extracts, considerable difference was found in the total phenolic and flavonoid contents (Table 3). The total phenolic content ranged from 3.81±1.37 to 341.99±4.00 mg GAE/ g extract. The *S. axillaris* contained the highest total phenolic content, followed by *M. edulis, D. butyracea* and *B. sapida* (Table 3).

The total flavonoid content ranged from 0.86±0.02 to 3.14±0.04 mg QE/g extract. The highest flavonoid content was found in *B. sapida* (3.14±0.04 mg QE/g extract) followed by *M. edulis* and *D. butyracea*. However, *S. axillaris* contained the lowest amount of flavonoid (Table 3). Earlier Singh *et al*.[25] have reported a significantly lower amount of phenolic content in these wild edible fruits.

Lycopene and β-carotene

Carotenoids are well known for their antioxidant properties[26]. It provides numerous health benefits to the human beings and decreases the chances of diseases with the main carotenoids being lycopene[27] and β-carotene[28]. The vital role of β-carotene is its provitamin A activity, and thus, it's deficiency can cause blindness, xerophthalmia and pre-mature death[29]. Lycopene does not convert to vitamin A but delay pathogenesis in several cancers and it also has antioxidant and antimutagenetic property[30].

The results obtained for the amount of β carotene and lycopene are depicted in Fig. 2. The mean lycopene content ranged from 3.00 -56.9 µg/100 g methanolic extract. *S. axillaris* contains the highest amount of lycopene whereas the minimum amount of lycopene was detected in *B. Sapida*. β-Carotene contents varied from 27.60 to 410.64 µg/100 g methanolic extract in the following order: *M. edulis*>*S. axillaris*>*D. butyracea*>*B. sapida*. β-Carotene is not only essential for vision improvement but also helps in proper immune functions, reproduction and scavenging of reactive oxygen species[31]. Based on the available literature, the present study reveals/analyses the lycopene and β-carotene content for the first time in wild edible fruits of Sikkim Himalaya.

Ascorbic acid content

The ascorbic acid content in the investigated fruits ranged between 631.90 and 780.90 mg/100 g methanolic extract of edible portion. *D. butyraceae* contains the highest amount of ascorbic acid. Other investigated fruits also contain substantial amount of ascorbic acid (Fig. 2). Ascorbic acid, well-known as vitamin C, is one of the most important antioxidants found in fruits[32]. It is essential for human balanced diet[33] and for the food industry as an additive of processed foods[34].

Antioxidant activity

The selected wild edible fruits were examined for their antioxidant property using two assays namely;

Table 3 — Extraction yield, total phenolic and flavonoid content of ethanolic extract of wild edible fruits.

Wild Edible Fruits	Extraction yield (% w/w)	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Baccaurea sapida	11.75±1.21b	3.81±1.37 d	3.14±0.04 a
Diploknema butyraceae	8.32±0.89c	12.01±0.97 c	1.25 ±0.32 c
Machilus edulis	19.85±1.40 a	80.55±6.63 b	1.78±0.09 b
Spondias axillaris	11.79±1.40 b	341.99±4.00 a	0.86±0.02 d

Mean values in each column followed by same lower-case letters are not significantly different separated using Duncan's multiple range test at $p<0.05$

DPPH and ABTS free radical assays and results are presented in Table 4. Different fruit extracts showed a significant variability in their inhibitory activity against DPPH and ABTS radicals. Among the investigated fruits, in both the assays, *S. axillaris* extract exhibited the highest scavenging activity, followed by *M. edulis* and *D. butyraceae*. Whereas, *B. sapida* exhibited the least antioxidant capacity. The ethanolic extract of *S. axillaris* showed even higher DPPH radical scavenging activity (IC_{50} value = 25.32±2.76 µg/mL) than the commercial antioxidant BHT (IC_{50} value = 46.53±0.19 µg/mL).

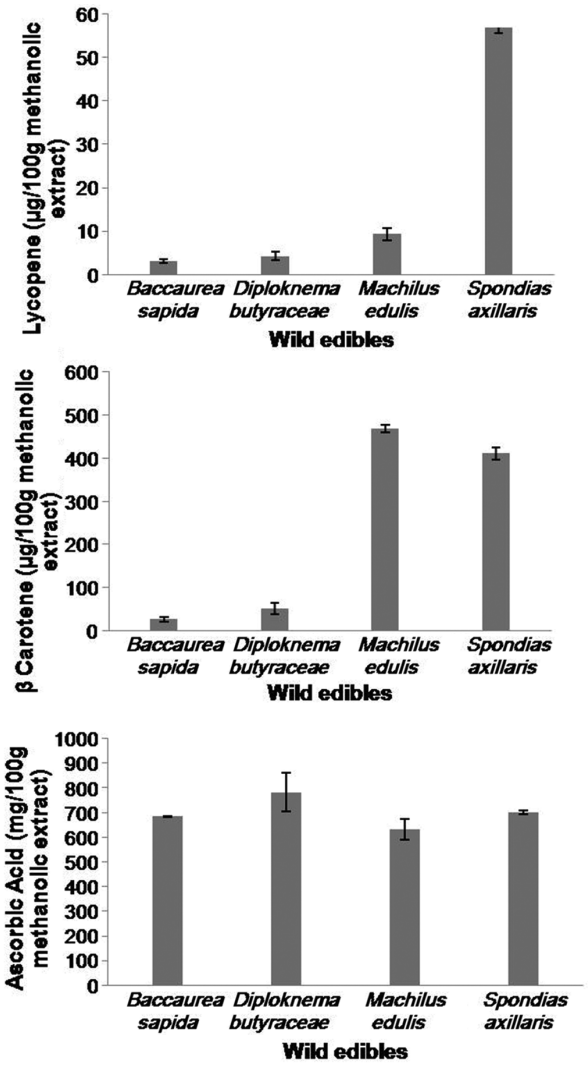

Fig. 2 — Lycopene, β carotene and ascorbic acid content in wild edible fruits.

Results of correlation analysis between the IC_{50} values for the DPPH and ABTS antioxidant assays and bioactive compounds showed a significant positive relationship between bioactive compounds and IC_{50} values. The total phenolic acids, lycopene and β -carotene showed negative correlations with the IC_{50} values ($p<0.05$) however, a significant positive correlation was observed among ascorbic acid and flavonoid content of fruits and the IC_{50} values. The results clearly indicate that the higher lycopene, β-carotene and total phenolic acids content corresponded to stronger antioxidant activities in wild edible fruits. These results are in line with the findings of previous studies that the antioxidant activity of fruits depends on the contents of lycopene, β-carotene and total phenolic acids[5,35,36].

Antioxidant compounds inhibit oxidation of important biomolecules through free radical-scavenging mechanisms[37]. Epidemiological researches recommend that higher intake of fruits and vegetables lowers the chances of various degenerative diseases[38] and decreases 14% cancer risk[39]. The modern lifestyle of human beings is leading to the increased formation of free radicals and reactive oxygen species, which causes the oxidative stress. The natural antioxidants play a significant role in healthcare by protecting from oxidative stress and related disorders[40]. The main sources of natural antioxidants are plant-derived foods, and fruits are the most important source of antioxidant compounds to humans[41]. Therefore, dietary use of such antioxidant-rich fruits can assist in improving the overall health of the rural peoples of the Himalayan region.

Table 4 — IC_{50} values of DPPH and ABTS activity of wild edible fruits.

Wild Edible Fruits	DPPH IC_{50} Value (µg/mL)	ABTS IC_{50} Value (µg/mL)
Baccaurea sapida	8724.33±633.24 a	407.20±34.00 a
Diploknema butyraceae	3960.00±158.00 b	89.24 ±0.63 b
Machilus edulis	140.64±3.25 c	6.63±0.08 c
Spondias axillaris	25.32±2.76 e	1.35±0.06 e
BHT	46.53±0.19 d	4.07±0.38 d

Mean values in each column followed by same lower-case letters are not significantly different separated using Duncan's multiple range test at $p<0.05$

Conclusions

The present investigation explored phytochemical and mineral contents of some high-value wild edible fruits of Sikkim Himalaya, in order to promote the consumption of wild edible fruits and to conserve their wild populations. Furthermore, the results indicated that these species are rich source of important antioxidants thus, can be utilized in the diet as nutraceuticals/ functional food for improving health and life quality of the Himalayan peoples. Among the investigated species, *S. axillaris* and *M. edulis* have shown the higher content of minerals, phenolics, β-carotene, and lycopene with strong antioxidant activity, therefore for sustainable utilization of natural resources, plantation of these high-value species in forest/wasteland is recommended at large scale to fulfill the nutritional requirement at commercial scale.

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Conflict of Interest

There are no conflicts of interest associated with this publication.

Authors' Contributions

MS conceptualize, carried out the research and analyzed the data. MS and AP wrote the MS and approved it

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