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Shagun Gupta
Assistant Professor, Department of Agriculture, SGRR University, Dehradun, Uttarakhand, India

Vibhuti Singh Jhala
Assistant Professor, Himalayan Institute of Pharmacy and Research, Dehradun, Uttarakhand, India

Chhaya Singh
Assistant professor, College of basic and applied sciences SGRR, University, Dehradun, Uttarakhand, India

Neha Chauhan
Dr. Neha Chauhan
Assistant Professor
SGRR College of Basic and Applied Sciences, SGRRU, Dehradun, Uttarakhand, India

Siddharth Shankar Bhatt
Assistant Professor, Department of Agriculture, SGRR University, Dehradun, Uttarakhand, India

Correspondence
Siddharth Shankar Bhatt
Assistant Professor, Department of Agriculture, SGRR University, Dehradun, Uttarakhand, India

Nutritional importance of underutilized fruits: '*Spondias axillaris* and *Eriolobus indica*' of Uttarakhand hills

Shagun Gupta, Vibhuti Singh Jhala, Chhaya Singh, Neha Chauhan and Siddharth Shankar Bhatt

Abstract
The present investigation revealed that the fruit of *S. axillaris* was found to be rich in dry matter (21.40±0.43%) crude protein (6.31±0.58%) and crude fat (2.10±0.28) as compared to *E. indica* (2.61±0.58%), whereas other nutritional component *viz.* fibre (1.55±0.30%), total carbohydrate (89.06±0.52) and energy value (393.14±1.96%) were higher in *E. indica.* Minerals like potassium was found to be highest in both the fruits i.e. *S. axillaris* (280.12 ± 6.58 µg/L) and E. *indica* (111.37 ± 4.38µg/L) and some other elements were also present significantly. Phytochemical *viz.* total phenol (71.83±0.76mg GAE/g) and ascorbic acid (34.54±0.99 mg/100 g) were found highest in *S. axillaris* while presence total flavonoid was maximum in E. *indica* (17.48±0.31 mg QE/ g). Anthocyanin and total carotenoid were also present in minute quantity in both the fruits. The study suggests that fruit of *S. axillaris* and *E. indica* could be good sources of nutrients, minerals and phytochemical and must be researched further for its beneficial effect in human health.

Keywords: Nutritional, phytochemical, minerals, total phenols, ascorbic acid, anthocyanin

1. Introduction
Fruit species, particularly those currently identified as 'underutilized', can contribute significantly to improve human health and nutrition, livelihoods, household food security and ecological sustainability which constitute an essential component in the diet of many ethnic population. The diversity of underutilized or lesser known plant species is very high (>250 species). Sundriyal *et al.*, (1998) [1] reported 190 fruit plant species that grow in the wild in. *S. axillaris* and *E. indica* are popular among them. *S. axillaris* is a wild large deciduous fruit tree belonging to Anacardiaceae family. Fruit is green in colour, turning yellow when ripe. Skin ripens to yellow with white flesh that has an acidic flavor. The mean dimensions of the fruit are about 25:3 mm x 22:4 mm x 22:6 mm. The mesocarp is pulpy. Each drupe contains a solitary brown seed with 4-5 depressions Bhutia, (2013) [2]. The fruits are rich in vitamin C content and are consumed fresh, pickled and processed into a variety of products. *S. axillar*ies have been reported to possess several properties for treatment of myocardial ischemia, calming nerves, ameliorating blood circulation and improving microcirculation in Mongolia Dai *et al.*, (1992) [3], Shi *et al.*,(1985) [4] Whereas, *E indica* is the Indian crab apple, belongs to the family Rosaceae, are round, pear shaped and pale green colour when ripe. They are eaten either fresh or processed into pickles as well as used in jelly preparation also. The fruit extract is made into a semi-solid gel locally known as 'chuk', which is considered to be a good medicine for stomach disorder De, (2017) [5]. Fruit extracts are traditionally being used for curing blood dysentery and bark used for piles. Therefore promoting the use of underutilized species needs to be achieved by emphasizing their nutritional and phytochemical significance.

2. Materials and Methods
The present investigation was carried out at Laboratory of Department of Agriculture, S. G. R. R. University during the year 2018. Experiment was conducted on two underutilized fruits grown naturally as forest vegetation of Himalayas were directly collected from the forest area of different region of Himalayas. Nutritional component *viz.* crude protein, crude fat, crude fibre, total carbohydrate, energy value, ash, moisture and dry matter content, vital elements and phytochemicals like total phenols, flavonoid, anthocyanin, Carotenoides and ascorbic were estimated using standard method of chemical analysis which are mentioned below:

2.1 Nutritional analysis
2.1.1 Moisture and Dry Matter Content
Moisture and Dry matter content were determined by following the method given by

(1990) [6]. Weighed sample (5.0 g) of each fresh fruit was taken in a sterilize weighed petri dish and kept in the hot air oven at 105°C for 12 hours and petri dish were then allowed to cool and weighed.The loss in weight represents the moisture content of the sample whereas, the dry matter content of the sample represents the amount of material left after the complete removal of moisture from the sample. The per cent moisture and dry matter content from the fruit sample were calculated by using the following formula: -

$$\text{Moisture content (\%)} = \frac{(\text{weight of fresh sample} - \text{weight of dry sample})}{\text{weight of fresh sample}} X\ 100$$

$$\text{Dry matter (\%)} = \frac{(\text{weight of petri dish} + \text{weight of dried sample}) - \text{weight of dish}}{\text{Weight of sample before drying}} X\ 100$$

2.1.2 Crude protein

The crude protein was estimated by Lowry's method by using UV/VIS Spectrophotometer, Perkin Elmer, Lambda 35 UV/VIS spectrometer suggested by Lowry, *et al.,* (1951)[7].

2.1.3 Crude fat

Crude fat content was determined by Soxhlet principle with slight modification A.O.A.C., (1990) [6]. Fat from the oven dried fruit sample was extracted in essential oil extractor (model no. Socsplus-SCS 06 DLS, PELICAN) using petroleum ether as solvent then ether is evaporated and determined the weight of the fat recovered using following formula:

$$\text{Crude fat (\%)} = \frac{(\text{W1} - \text{W2})}{\text{weight of sample}} X\ 100$$

2.1.4 Crude fibre

Crude fibre was analyzed using fibre estimation system, model no Fibra plus-FES 04 AS DLS, PELICAN.2 g of moisture and fat free sample were taken in the crucibles then it was loaded in the instrument. 150 ml of 1.25% of H_2SO_4 was added from the top and boiled at 500° C for 30 minutes. Once the boiling was completed the reagents was drained out with the help of fibra flow then 150 ml of 1.25% NaOH was added from the top and heating the sample at 400° C for 45 minutes which led to digestion of sample. After completion of digestion reagents was drained out and residue was dried in hot air oven at 90 -100 °C and cooled and weighed the dried residue (W_1) then the residue was kept in pre-weighed porcelain crucible and put in the muffle furnace for ashes at 600°C in 3 hours then it was cooled and weighed (W_2). Crude fibre content was expressed as percentage loss in weight on ignition A.O.A.C., (1990) [6] and calculated using following formula:

$$\text{Crude Fibre (\%)} = \frac{(\text{W1} - \text{W2})}{\text{weight of sample}} X\ 100$$

2.1.5 Ash content

Ash content was determined by following the method of A.O.A.C., (1990) [6]. Crucible were kept in a muffle furnace at 600^0C for 1h. Then they were transferred from furnace and cooled to room temperature and weighed (W_1) as quickly as possible to prevent moisture absorption. 2 g dried fruit sample was taken in crucible and placed in a muffle furnace at 600°C for 6h. Then crucible was transferred to get cooled at room temperature and weighed (W_2). Then the percentage of ash was calculated by using the following formula

$$\text{Ash (\%)} = \frac{\text{W1} - \text{W2 (weight of ash)}}{\text{weight of sample}} X\ 100$$

2.1.6 Available carbohydrate

The percentage of available carbohydrate was calculated by: 100- (Percentage of ash+ Percentage of fat + Percentage of fibre + Percentage of protein) method suggested by A.O.A.C., (1990) [6].

2.1.7 Energy value/nutritive value

The energy value in kilocalorie per gram (Kcal/g) was determined by multiplying the percentage of crude proteins, crude fat and carbohydrate by the recommended factor 4, 9 and 4, respectively and then taken the sum of values. The value was then converted to kilojoules by multiplying with 4.2 method suggested by A.O.A.C., (1990) [6].

Energy value (Kcal/g) = (CP x 4) + (CF x 9) + (Carb. x 4)

2.2 Mineral Analysis

ICP-MS (Inductively Coupled Plasma Mass Spectrophotometry) Perkin Elmer Nex ION 300X was used for estimation of some mineral elements. Digested samples were analyzed for the ionic constitution using multi elements standards for detecting the elements such as Ca, Fe, Mg, Mn, Mo, Na, Zn. The micro wave digestion system (Anton par microwave 3000) was used for sample digestion as 0.5 gm sample were along 9ml of 69% nitric acid and 2ml HCl were added into the digestion tube and run the instrument for 40 minutes. The digested samples were then transferred into 50ml volumetric flask when the temperature of the sample was reduced and distilled water was added for making the volume of 50 ml. The liquid sample was transferred into narrow mouth bottle until the minerals were determined in ICP-MS. The values of the elements were expressed as µg/L.

2.3 Phytochemical analysis

Extraction of fruit sample: The matured fruits of *S. axillaris* and *E. indica* were collected from different places were washed and cleaned thoroughly in running water. Fruits were then chopped into small pieces and dried at 105 ° C for 48 hours in hot air oven. Dried sample were then grind into fine powder using Willey mill and 5 gram of sample each sample was extracted using 50 ml solvent (80% methanol) for 12 hours at 60° C temperature in Soxhlet apparatus (essential oil extractor: model no. Socsplus-SCS 06 DLS, PELICAN). After completion of boiling, temperature was increased to 150 ^{0}C for 45 min to evaporate the solvent. The extract were concentrated to dryness in rotary evaporator under reduced pressure and weighed. The extracts were then diluted with known volume (mg/ml) of methanol in air tight small container and kept under refrigerator at 4 °C until analysis.

2.3.1 Total phenols

The concentrations of total phenol content of methanol extract of fruits were determined in UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer) by employing the method given by Singleton, *et al.,* (1999) [8] with minor modification involving Folin-Ciocalteau Reagents as oxidizing agent and Gallic acid as standard.

2.3.2 Total Flavonoid

The aluminum chloride assay was used for the determination of the total flavonoid content of the fruit extracts according to the method described by Kumaran and Karunakaran, (2007)

[9] with slight modifications in UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer).

2.3.3 Ascorbic acid

The ascorbic acid was determined by reduction of 2, 6-dichlorophenol indophenols dye by ascorbic acid as procedure A.O.A.C., (1980) [10]. Ten (10) ml of juice was taken and blended with 0.4% HPO_3 and finally volume was made up to 100 ml with 0.4% HPO_3 and then 10 ml aliquot was titrated against standardized dye to obtain a pink colour which persists at least for 15 seconds. Ascorbic acid was expressed in terms of mg per 100 gm pulp by using formula:

$$\text{Ascorbic acid (mg/100 g pulp)} = \frac{\text{Dye factor x titre reading x dilution}}{\text{Weight of sample}} \text{x100}$$

2.3.4 Anthocyanin content

Anthocyanin content was determined by the method described by Srivastava, *et al.,* (2003) [11] with some modification. Sample was extracted by blending 10 g of finely ground sample with 10 ml of 95% ethenolic HCL and centrifuged at 10000 rpm for 20 minutes then supernatant was collected and transferred into 100 ml volumetric flask and volume was made up to the mark and solution was stored in the refrigerator at 4° C until analysis. The optical density of the aliquot was determined at 530 nm in UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer). The value of total anthocyanin content was expressed as mg/100 gram. Calculation was done by using the following formula.

$$\text{Total O.D/100g} = \frac{\text{O.D X volume made up x100}}{\text{Weight of sample}}$$

$$\text{Total Anthocyanin (mg/100g)} = \frac{\text{total O.D/100}}{\text{Weight 98.2 of sample}}$$

2.3.5 Total carotenoids

One gram of sample was weighed and grinds it with acetone using acid and alkali washed sand in a pestle and mortar. The extract is decanted into a conical flask. Continue the extraction till the residue was colorless. The acetone extract was transferred to a separating funnel containing 10-15 ml of petroleum ether and mixed gently. About 25 ml of 5% sodium sulphate solution was added. Shaken and kept for sometimes and yellow colour pigment is transferred into the petroleum ether later. Collected the layer in a volumetric flask and separated acetone layer containing 5% sodium sulphate. Keep on adding 15 ml petroleum ether to the acetone layer containing Na_2So_4 until the colour gets transferred into the petroleum ether and measured the colour intensity at 452 nm in a spectrophotometer. And the total carotenoids content was calculated using the following formula:

$$\text{Total catotenoids (mg/100 g)} = \frac{3.857 \times \text{O.D} \times \text{Volume made up} \times 100}{\text{Weight of the sample} \times 1000}$$

2.3.6 Statistical analysis

All the experiments were carried out in triplicates and data were expressed as mean ± standard deviation.

3. Results and Discussion

3.1 Nutritional compositions

The result of the nutritional assessment shown in table 1 indicate that the fruit of *S. axillaris* contains 78.70±0.48% moisture and 21.40±0.43% dry matter, 6.31±0.58% crude protein, 2.10±0.28% crude fat, 1.55±0.30% fibre, 2.89±0.28% ash, 87.07±0.01% total carbohydrate and 373.18±2.28 kcal/100 g energy value, whereas, fruit of *E. indica* was found to contain 86.03±0.06% moisture and 13.91±0.11% dry matter content, 2.61±0.58% crude protein, 0.87±0.02% fat, 4.57±0.06% fibre, 2.90±0.49% ash, 89.06±0.52% carbohydrate and 393.14±1.96 Kcal/100 g of energy value. The results of nutritional assessment revealed that both the fruits were good source of nutrition. Fruits of S. *axillaris* were found to contain higher amount of dry matter, protein and fat whereas, fruit of *E. indica* were rich in moisture, fibre, ash, total carbohydrate and energy value. Seal *et al.,* (2014) [12] results also indicated that the fruit of *S. axillaris* was found to contain 62.29% of moisture which was lesser than our findings. Rai *et al.,* (2005) [13] reported 85.1% moisture content in *E. indica* which was similar to our present finding. Kumar *et al.,* (2015) [14] also noted the protein content in a range of 2-10% while studying of underutilized fruits. Seal *et al.,* (2014) [12] results also showed appreciable amount of protein (1.88%), fat (7.39%), fibre (9.35%) and ash (3.53%) in the fruit of *S. axillaris.* Fibre is also one of the major component of nutritional composition known to reduce risk of some of the world's most prevalent disease like obesity, diabetes, high blood cholesterol, cardiovascular disease, and numerous gastrointestinal disorders Venn and Mann, (2004) [15], Tungland and Meyer, (2002) [16].Total carbohydrate content of *Eriolobus indica* (71.73%) and *Spondias axillaris* (52.28%) was also reported by Sundriyal and Sundriyal, (2001) [17].

Table 1: Nutritional composition of fruit of the *Spondias axillaris* and *Eriolobus indica*

Sl. No.	Nutritional content (%)	*Spondias axillaris*	*Eriolobus indica*
1	Moisture	78.70±0.48	86.03±0.06
2	Dry matter	21.40±0.43	13.91±0.11
3	Crude protein	6.31±0.58	2.61±0.58
4	Crude fat	2.10±0.28	0.87±0.02
5	Fiber	1.55±0.30	4.57±0.06
6	Ash	2.89±0.28	2.90±0.49
7	Total carbohydrate	87.07±0.01	89.06±0.52
8	Energy value (kcal/100 g)	373.18±2.28	393.14±1.96

3.2 Mineral compositions

The results of the mineral profiling are presented in table 2. The assessment of mineral composition of the two different underutilized in present study revealed that the fruit of *S. axillaris* contains Ca (12.37 ± 1.9µg/L), Mg (40.16 ± 1.9µg/L), K (280.12 ± 6.58µg/L), Mo (2.63 ± 0.8µg/L), Na (6.96 ± 1.3µg/L), Zn (0.11 ± 0.08µg/L), Fe (26.47 ± 2.4µg/L), Co (9.06 ± 1.8µg/L) and Mn (11.57 ± 1.2µg/L). Whereas, the fruit of *E. indica* measured Ca (1.98 ± 0.02µg/L), Mg (6.88 ± 0.8µg/L), K (111.37 ± 4.38µg/L), Mo (0.79 ± 0.05µg/L), Na (7.05 ± 1.4µg/L), Zn (0.16 ± 0.2µg/L), Fe (23.29 ± 2.1µg/L), Co (4.16 ± 0.9µg/L) and Mn (3.09 ± 0.18µg/L). Current research finding pertaining to mineral component showed maximum amount of potassium in both the fruits while other minerals were found in minute quantity. Kalita *et al.,* (2014) [18] evaluated the nutritional potential of five unexplored wild edible food plants from eastern Himalayan biodiversity hotspot region (India) and significant variation among the mineral compositions was noticed as potassium and phosphorus were the most abundant of the elements considered followed by calcium and sodium. Seal *et al.,* (2014) [12] results depictedthe fruit of *S. axillaris* was found to

contain varying concentration of minerals *viz.* sodium (0.81 mg/g), potassium (10.81 mg/g), calcium (6.05 mg/g), manganese (0.05 mg/g), magnesium (0.85 mg/g), iron (0.37 mg/g), zinc (0.30 mg/g) and copper (0.052 mg/g) and the concentration of potassium was reported to be highest among other minerals. Potassium is one of the most essential and major plant nutrients and foods rich in potassium are generally used for the treatment of rheumatoid arthritis and heart disease reported by Borah *et al*, (2009) [19].

Table 2: Mineral contents of *Spondias axillaris* and *Eriolobus indica*

Sl. No.	Mineral content (µg/L)	*Spondias axillaris*	*Eriolobus indica*
1	Calcium	12.37 ± 1.9	1.98 ± 0.02
2	Magnesium	40.16 ± 1.9	6.88 ± 0.8
3	Potassium	280.12 ± 6.58	111.37 ± 4.38
4	Molybdenum	2.63 ± 0.8	0.79 ± 0.05
5	Sodium	6.96 ± 1.3	7.05 ± 1.4
6	Zinc	0.11 ± 0.08	0.16 ± 0.2
7	Iron	26.47 ± 2.4	23.29 ± 2.1
8	Copper	9.06 ± 1.8	4.16 ± 0.9
9.	Manganese	11.57 ± 1.2	3.09 ± 0.18

3.3 Phytochemical constituents

The results of the phytochemical constituents are presented in table 3. Methanol extracts of *S. axillaris* and *E. indica* fruits were used to estimate phytochemical content *viz.* total phenols, total flavonoids, anthocyanin, total carotenoids and ascorbic acid. The present finding revealed that the fruits of *S. axillaris* were good source of total phenols as it contains 71.83±0.76 mg GAE/g followed by ascorbic acid 34.54±0.99 mg/100 g and total flavonoids (7.83±0.17 mg QE/g). Anthocyanin and total caretonoids were also detected in small quantity i.e. 0.74±0.02mg/ 100g and 0.9±0.3 mg/100 g, respectively. The fruits of *E. indica* also contained17.48±0.31 mg QE/ g total flavonoids, 12.29±0.30 mg GAE/g of total phenols, 9.7±0.59 mg/100 g ascorbic acid, 1.33±0.73 mg/100 g anthocyanin and 0.99±0.09 mg/100 g total carotenoids. Singh *et al.*, (2014) [20] studied the phenolic content and antioxidant activity of some underutilized wild edible fruits and value of total phenolic content of different fruits were reported as highest in *Spondias axillaris* (69. 4 mg GAE/g)and lowest in *Eriolobus indica* (10 mg GAE/g) which were similar to our findings. Prakash *et al.*, (2012) [21] found 69.4 mg/g of total phenol in the fruit of *S. axillaris*and appreciable amount of TPC was also noted in the fruit of *E. indica* (23.7 mg GAE/g).

Table 3: Phytochemical content of *Spondias axillaris* and *Eriolobus indica*

Sl. No.	Phytochemicals	*Spondias axillaris*	*Eriolobus indica*
1	Total phenols (mg GAE/g)	71.83±0.76	12.29±0.30
2	Total flavonoid (mg QE/ g)	7.83±0.17	17.48±0.31
3	Anthocyanin (mg/ 100g)	0.74±0.02	1.33±0.73
4	Total Carotenoides (mg/100 g)	0.9±0.3	0.99±0.09
5	Ascorbic acid (mg/100 g)	34.54±0.99	9.7±0.59

4. Conclusion

There is emerging concern about the food related issues to human health and also the world is expected to produce more food to meet the demand of growing population. In that case underutilized fruits and other edible resources are gaining popularity. The underutilized fruit resources have more nutritional value than the other known species. The present research finding also depicts that the fruit of *S. axillaris* and *E. indica* were found to be good source of nutrients, minerals and phytochemicals which can add value to human diet and they can be further explored for future research and product developments or industrial purpose.

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