Screening of nutritional composition and phytochemical content of underutilized fruits – ‘Spondias axillaris and Eriolobus indica’ of Sikkim Himalayas.

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Abstract
The purpose of present study was to investigate the nutritional composition, mineral profile and phytochemical content of two underutilized fruits ‘Spondias axillaris and Eriolobus indica’ of Sikkim Himalaya. 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
Sikkim is a North Eastern state of India having small geographical area of 7096 sq. km. situated at latitude 27° – 28° N, and longitude of 88° – 89° E. The climate of the state has been roughly divided into the tropical, subtropical, temperate and alpine zones. The state is blessed with abundant natural resources which have been used by people for many years in different ways. 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) in the Sikkim Himalaya. Sundriyal et al., (1998) [1] reported 190 fruit plant species that grow in the wild in Sikkim. 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. axillaris 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, locally known as Mehl in Nepali (Sikkim) and is commonly found in the region particularly in Sikkim, Darjeeling and Meghalaya. The fruits 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’ in Sikkim, 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 entitled ‘Nutritional composition and phytochemical screening of *Spondias axillaris* and *Eriolobus indica* fruits of Sikkim Himalayas’ was carried out at Laboratory of Department of Horticulture, Sikkim University during the year 2014-2017. Experiment was conducted on two underutilized fruits grown naturally as forest vegetation of Sikkim Himalayas were directly collected from the forest area of different region of Sikkim. 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 A.O.A.C., (1990) [6]. Weighed sample (5.0 g) of each fresh fruit was taken in a sterile 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:

\[
\text{Moisture content} = \frac{(\text{weight of fresh sample - weight of dry sample})}{\text{weight of fresh sample}} \times 100
\]

\[
\text{Dry matter} = \frac{(\text{weight of petri dish + weight of dried sample}) - \text{weight of dish}}{\text{Weight of sample before drying}} \times 100
\]

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. Socplus-SCS 06 DLS, PELICAN) using petroleum ether as solvent then ether is evaporated and determined the weight of the fat recovered using following formula:

\[
\text{Crude fat} = \frac{(W1 - W2)}{\text{weight of sample}} \times 100
\]

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_{2}SO_{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 (W1) 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 (W2). Crude fibre content was expressed as percentage loss in weight on ignition A.O.A.C., (1990) [6] and calculated using following formula:

\[
\text{Crude Fibre} = \frac{(W1 - W2)}{\text{weight of sample}} \times 100
\]

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°C for 1h. Then they were transferred from furnace and cooled to room temperature and weighed (W1) 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 (W2). Then the percentage of ash was calculated by using the following formula:

\[
\text{Ash} = \frac{W1 - W2 (weight of ash)}{weight of sample} \times 100
\]

2.1.6. Available carbohydrate

The percentage of available carbohydrate was calculated by:

\[
\text{100 - (Percentage of ash+ Percentage of fat + Percentage of fibre + Percentage of protein)}
\]

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 of Sikkim 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 Wilemy 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° C for 45min 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.

\[
\text{Ascorbic acid (mg/100 g pulp)} = \frac{\text{Dye factor} \times \text{titre reading} \times \text{dilution}}{\text{Weight of sample}} \times 100
\]

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 % ethanolic 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.

\[
\text{Total O.D/100 g} = \frac{\text{O.D} \times \text{volume made up} \times 100}{\text{Weight of sample}}
\]

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 Na₂SO₄ 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:

\[
\text{Total carotenoids (mg/100 g)} = \frac{3.857 \times \text{OD} \times \text{X Volume made up} \times 100}{\text{Weight of the sample} \times 1000}
\]

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

4. Results and Discussion
4.1. 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], Sungland 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] while studying the fruits from Sikkim Himalaya.
4.2. Mineral compositions
The results of the mineral profiling are presented in table 2. The assessment of mineral composition of the two different underutilized fruit from Sikkim Himalayas in present study revealed that the fruit of S. axillaris contains Ca (12.37 ± 1.9 μg/L), Mg (40.16 ± 1.9 μg/L), K (280.12 ± 6.58 μg/L), Mo (2.63 ± 0.8 μg/L), Na (6.96 ± 1.3 μg/L), Zn (0.11 ± 0.08 μg/L), Fe (26.47 ± 2.4 μg/L), Co (9.06 ± 1.8 μg/L) and Mn (11.57 ± 1.2 μg/L). Whereas, the fruit of E. indica measured Ca (1.98 ± 0.02 μg/L), Mg (6.88 ± 0.8 μg/L), K (111.37 ± 4.38 μg/L), Mo (0.79 ± 0.05 μg/L), Na (7.05 ± 1.4 μg/L), Zn (0.16 ± 0.2 μg/L), Fe (23.29 ± 2.1 μg/L), Co (4.16 ± 0.9 μg/L) and Mn (3.09 ± 0.18 μg/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 depicted the 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

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

4.2. 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 carotenoids were also detected in small quantity i.e. 0.74±0.02 mg/ 100g and 0.92±0.3 mg/100 g, Respectively. The fruits of E. indica also contained 17.48±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, 1.3±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 of Sikkim Himalaya 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 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

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Phytochemicals</th>
<th>Spondias axillaris</th>
<th>Eriolobus indica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total phenols (mg GAE/g)</td>
<td>71.83±0.76</td>
<td>12.29±0.30</td>
</tr>
<tr>
<td>2</td>
<td>Total flavonoid (mg QE/g)</td>
<td>7.83±0.17</td>
<td>17.48±0.31</td>
</tr>
<tr>
<td>3</td>
<td>Anthocyanin (mg/100g)</td>
<td>0.74±0.02</td>
<td>1.3±0.73</td>
</tr>
<tr>
<td>4</td>
<td>Total carotenoides (mg/100g)</td>
<td>0.9±0.3</td>
<td>0.99±0.09</td>
</tr>
<tr>
<td>5</td>
<td>Ascorbic acid (mg/100g)</td>
<td>34.54±0.99</td>
<td>9.7±0.59</td>
</tr>
</tbody>
</table>

5. 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.

6. Acknowledgements
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7. Reference