



Determination of Nutritional Content of *Spondias* Species from the Eastern Himalaya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out with the aim to screen out the nutritional characteristics of three underutilized fruit species of the genus *Spondias* which are seen grown wild in the forest of the north-eastern region of India, as the detail information on nutrient composition of these fruit species is scarce and people are unaware of the nutritional factor though inadvertently consuming a good amount of nutrients from these fruits. The present nutritional assessment revealed that the fruit of *Spondias pinnata* was found to possess highest TSS content (15.27 °Brix) however it was observed to exhibit higher acidity (4.59%) as well. The minimum titratable acidity was found in *Spondias axillaris* (2.45%). *Spondias pinnata* exhibited the highest ascorbic acid content (74.16 mg/100 g) followed by *Spondias axillaris* (61.60 mg/100 g) while the lowest (31.55 mg/100 g) was found in *Spondias cytherea*. Further, from the experiment, it was found that *Spondias pinnata*

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having the highest ascorbic acid was also found to exhibit highest DPPH free radical scavenging activity (68.49%). *Spondias pinnata* was also found to possess maximum reducing sugar (7.32%), total carbohydrate (12.51%), total chlorophyll (0.03 mg/g), total carotenoid (1.30 mg/100 g) and starch content (195.72 mg/100 g). As far as total free amino acid was concern, *Spondias cytherea* recorded highest total free amino acid content (158.67 mg/100 g) showing a wide variation in comparison to *Spondias axillaris* (25.33 mg/100 g) and *Spondias pinnata* (22.67 mg/100 g). These wild fruit species under study proved to be a good source of nutrients with a potential to fulfill the nutritional requirements locally.

Keywords: Underutilized fruits; *Spondias* species; nutritional; North-Eastern Region; India.

1. INTRODUCTION

The North-eastern region of India, a part of the eastern Himalayan region lying between 21.5° N - 29.5° N latitudes and 85.5° E - 97.3° E longitudes spreading over an area of 2,620,230 sq.km (8% of country's geographical area) comprises of eight states - Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. The region, due to high rainfall, humidity and diverse climate coupled with varying altitude, topography and soil status provides a variety of microclimates and ecological niches which favours high species richness and makes it unique to grow large number of underutilized fruit crops. It is a region of active speciation and has been designed as cradle of flowering plants [1]. This region is the storehouse of many wild relatives of underutilized fruits of potential importance of which many are endemic in nature [2].

Underutilized fruit crops in the region play a vital role in the life of rural people from various angles. Many of them have medicinal, therapeutic, commercial values and most importantly contribute food and nutritional security by forming an integral part of dietary system in the rural areas of the region. Among different underutilized fruits species found, three species of genus *Spondias* viz. *Spondias pinnata*, *Spondias axillaris* and *Spondias cytherea* belonging to the family Anacardiaceae are seen grown in the wild and backyard of the region. However, these fruit species remain largely underutilized and people are unaware of the nutritional factor though inadvertently consuming a good amount of nutrients from these fruits.

Spondias pinnata L. also known as Indian hog plum, is a deciduous, medium sized glabrous tree up to 10.5 m high with straight trunk having a pleasant aromatic acidic smell. Leaves are glossy and compound, leaflets opposite, 3 paired. Fruit is drupe more than an inch long,

ovate, elliptic in shaped, acidic, aromatic, greenish yellow when ripe and bear in cluster during winter months.

Spondias axillaris Roxb. also known as Nepali hog plum, is a wild large deciduous fruit tree. Leaves are petiolulate, imparipinnately compound with opposite leaflets. The midribs of juvenile leaves displayed red-brown to red-orange colouration. Fruit is green in colour, turning yellow when ripe. Skin ripens to yellow with white flesh that has an acidic flavor. Fruit is a drupe, ellipsoidal or spherical. The mesocarp is pulpy and slippery. Each drupe contains a solitary brown seed with 4-5 depressions [3].

Spondias cytherea Sonn. also known as Ambarella or the golden apple is a fast growing tree up to 30-40 ft high, upright, rigid. Pinnate leaves, 8-24 inches in length composed of 9-25 glossy, elliptic or obovate-oblong leaflets finely toothed towards the apex. Long stalked fruits dangled in bunch of a dozen or more, oval or somewhat irregular or knobby. The flesh is crisp, juicy and sub-acid when fruits are still green. At ripe stage, the flesh becomes difficult to slice because of tough fibres extending from the rough ridges of the 5 celled, woody core containing 1-5 flat seeds.

As there are not much record on the nutritional chemistry and attributes of these underutilized fruit species. Therefore, it is imperative and essential to carry out studies on nutritional profiling of these fruit species and execute to explore the hidden potential of these novel fruits to strengthen the security in terms of food and nutrition by developing a food composition database. Considering the above gap, the present investigation was carried out to study the nutritional composition of these three *Spondias* fruit species.

2. MATERIALS AND METHODS

The present work on nutritional profiling of fruits of *Spondias* species was carried out under the

Department of Fruit Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat, East Siang district, Arunachal Pradesh during the year 2017-2020. Three *Spondias* species viz. *Spondias pinnata*, *Spondias axillaris* and *Spondias cytherea* were selected for the study. Ripe fruits were collected from the forest area of different states of the North-eastern region along with the GPS coordinates (Table 1). Respective fruit samples were collected randomly from all direction of the tree and pooled to form a single sample. Further, this fruit lot was divided into three replications for estimation of nutritional compositions on fresh weight basis.

Nutritional components viz. TSS, titratable acidity, reducing sugar, total carbohydrate, ascorbic acid, DPPH free radical scavenging activity, starch, total chlorophyll, total carotenoid and total free amino acid were estimated using standard method of chemical analysis.

Total soluble solids (TSS) was determined by one drop of the juice calibrated in digital refractometer.

Titratable acidity of the fruit was determined by titrating the fruit juice against 0.1 N NaOH solution using phenolphthalein as an indicator (light pink end point) and expressed as percentage in terms of citric acid [4].

Acidity (%) =

$$\frac{\text{Titre reading} \times \text{Normality of alkali} \times \text{Equivalent weight of acid} \times 100}{\text{Volume of sample taken}}$$

Reducing sugar was estimated by Nelson-Somogyi method as described by Sadasivam et al. [5]. Here, sugar was extracted by macerating 100 mg of the sample with 5 ml of warm 80 per cent ethanol. The content was centrifuged for 5 minutes. Water was added to dissolve the sugars. An aliquot of 0.2 ml was taken and diluted to 2 ml with distilled water. Further, 1 ml of alkaline copper tartrate reagent was added to each tube. The tube was placed in boiling water for 10 minutes. The tube was allowed to cool down and 1 ml of arsenomolybdic acid reagent

was added to each tube, final volume was made up to 10 ml with distilled water. The content was incubated for 10 minutes at room temperature. After 10 minutes the sample was read against the blank solution in a UV visible spectrophotometer at 620 nm.

Total carbohydrate was estimated by Anthrone method as described by Sadasivam et al. [5]. Sample (100 mg) was taken in a boiling tube and hydrolysed by keeping it in a water bath for three hours with 5 ml of 2.5 N HCl. The sample was cooled to room temperature and was neutralized with sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and the content was centrifuged and supernatant was collected. An aliquot of 1 ml was taken and 4 ml of anthrone reagent was added to it. The content was heated for eight minutes in boiling water bath and was cooled rapidly. Dark green colour of the sample was read against the blank solution at 630 nm using UV visible spectrophotometer.

Ascorbic acid content was determined by the method described by Ranganna [6]. 5 g of sample was taken, blended with 3 per cent metaphosphoric acid and made the volume to 50 ml with metaphosphoric acid and filtered. Then titrated 5 ml aliquot with standard dye to a pink colour end point which persisted for at least 15 seconds. Ascorbic acid was calculated as:

Ascorbic acid (mg/100 g) =

$$\frac{\text{Titre value} \times \text{Dye factor} \times \text{volume made up} \times 100}{\text{volume of aliquot taken for estimation} \times \text{weight of sample}}$$

DPPH free radical scavenging activity was determined according to the method of Aoshima et al. [7]. Sample (200 mg) was homogenized in 5 ml of absolute ethanol. Briefly to 0.5 ml of sample extract, 0.3 ml of DPPH reagent was added and vortexed vigorously. The reaction mixture was stored in the dark for 30 minutes at room temperature and discolouration of DPPH was measured against a blank at 517 nm using an UV visible spectrophotometer.

Table 1. Source of collection

Species	Family	Source of collection	Latitude	Longitude	Altitude
<i>Spondias pinnata</i>	Anacardiaceae	Dimapur district, Nagaland	25°50'16.06"N	93°45'42.77"E	171 m
<i>Spondias axillaris</i>	Anacardiaceae	South Sikkim district, Sikkim	27°17'4.59"N	88°19'23.94"E	1590 m
<i>Spondias cytherea</i>	Anacardiaceae	West Tripura district, Tripura	23°54'12.95"N	91°18'55.14"E	54 m

Starch content was determined by the method of Hedge and Hofreiter [8]. Sample (500 mg) was homogenized in 80% warm ethanol to remove the sugars and the content was centrifuged and residue was collected. The residue was repeatedly washed with 80% warm ethanol. The residue was dissolved in 5 ml of water and 6.5 ml of 52% perchloric acid was added to it and the content was incubated at 0°C for 20 minutes. The content was centrifuged and the supernatant was collected. Further, 0.2 ml of the supernatant was pipetted out and the volume was made up to 1 ml with distilled water followed by addition of 4 ml of anthrone reagent to it. The content was then heated in boiling water bath for 8 minutes and cooled rapidly. The intensity of dark green colour was read against the blank solution at 630 nm using an UV visible spectrophotometer.

Chlorophyll content was determined by the method described by Arnon [9]. Sample (100 mg) was homogenized with 20 ml of extractant solvent (80 per cent acetone). Sample was centrifuged at 5000 rpm for 5 minutes at 4°C and supernatant was collected. Repeat the procedure until the residue become colourless. Then the volume was made up to 100 ml with 80 per cent acetone. Sample was read in UV visible spectrophotometer at two different wavelengths 663 nm and 645 nm.

The estimation of carotenoid content was determined by the method described by Lichtenthaler [10]. Sample (100 mg) was homogenized with 5 ml of 80 per cent acetone. After centrifuging at 5000 rpm for 5 minutes, the supernatant was collected and used to determine the total carotenoids by recording the absorbance at 664, 648 and 470 nm using UV visible spectrophotometer.

Total free amino acid content was determined by the method described by Moore and Stein [11]. The sample (500 mg) was ground with 10 ml of 80 per cent ethanol. The content was filtered and filtrate was collected. An aliquot of 0.1 ml was taken and 1 ml of ninhydrin solution was added and was diluted to 2 ml with distilled water. Tube was placed in boiling water bath for 20 minutes. Tube was allowed to cool down and 5 ml of diluent solvent was added to each tube. After 15 minutes sample was read against the blank solution in UV visible spectrophotometer at 570 nm.

The experiment measurements were carried out in triplicates and the data were expressed as mean \pm standard deviation.

3. RESULTS AND DISCUSSION

The results of the nutritional assessment are enumerated in Table 2 which indicated that *Spondias pinnata* recorded the highest TSS (15.27 °Brix). However it was observed to exhibit higher acidity (4.59%). The soluble solids in fruit pulp of *Spondias pinnata* was found in close range to the current study as confirmed by a team of researchers [12]. The higher TSS may be due to the fact that since the fruit tree is grown under natural water scarce condition without care and management it tends to accumulate more dry matter eventually increasing the TSS content [13].

The minimum titratable acidity was found in *Spondias axillaris* (2.45%). The value of acidity in *Spondias axillaris* is in accordance to the range reported by some workers [14,15].

As such, ascorbic acid is considered as one of the popular antioxidants, which play a vital role in preventing peroxidation damage in the biological systems [16]. Being a strong reducing agent, it helps to tie up free radicals and thus protect the body from their deleterious effects [17], apparently, signifying that fruits possessing good source of vitamin C are a strong antioxidant. In the current study, *Spondias pinnata* exhibited the highest ascorbic acid content (74.16 mg/100 g) followed by *Spondias axillaris* (61.60 mg/100 g) while the lowest (31.55 mg/100 g) was found in *Spondias cytherea*. Further, the radical scavenging activity of the studied fruits was in the order of *Spondias pinnata* > *Spondias axillaris* > *Spondias cytherea*. From the experiment, it was found that *Spondias pinnata* having the highest ascorbic acid was also found to exhibit highest DPPH free radical scavenging activity (68.49%). These may be attributed to the presence of some other antioxidant phytochemicals [18]. Similar findings on *Spondias pinnata* having high content of ascorbic acid with good antioxidant activity was earlier reported [19].

Spondias pinnata was also found to possess highest reducing sugar (7.32%) and total carbohydrate (12.51%) meanwhile *Spondias axillaris* exhibited lowest reducing sugar (0.80%) and total carbohydrate (1.75%) respectively. Reducing sugar and total carbohydrate content in *Spondias pinnata* is in line with the findings reported by some research workers [20].

Table 2. Nutritional compositions of *Spondias* species

Parameters	Species		
	<i>Spondias pinnata</i>	<i>Spondias axillaris</i>	<i>Spondias cytherea</i>
TSS (°Brix)	15.27 ± 0.17	10.57 ± 0.33	11.00 ± 0.41
Titrateable acidity (%)	4.59 ± 0.54	2.45 ± 0.15	2.77 ± 0.54
Ascorbic acid (mg/100 g)	74.16 ± 5.33	61.60 ± 5.03	31.55 ± 3.43
DPPH free radical scavenging activity (%)	68.49 ± 0.41	66.29 ± 0.23	58.61 ± 1.38
Reducing sugar (%)	7.32 ± 0.35	0.80 ± 0.04	3.19 ± 0.07
Total carbohydrate (%)	12.51 ± 0.22	1.75 ± 0.14	7.28 ± 0.13
Total chlorophyll (mg/g)	0.03 ± 0.008	0.03 ± 0.005	0.01 ± 0.003
Total carotenoid (mg/100 g)	1.30 ± 0.14	0.34 ± 0.10	0.17 ± 0.03
Starch (mg/100 g)	195.72 ± 6.18	85.26 ± 7.00	93.87 ± 2.57
Total free amino acid (mg/100 g)	22.67 ± 4.99	25.33 ± 4.99	158.67 ± 6.80

All values are expressed as mean ± standard deviation of triplicate of three biological sample determinations

With respect to total chlorophyll and total carotenoid contents, *Spondias pinnata* showed a higher value with (0.03 mg/g) and (1.30 mg/100 g) respectively. The carotenoid content in the fruit pulp of one of the *Spondias* species was found slightly higher to the present work who obtained a value of 2.08 mg/100 g [21].

Starch content showed variation among the species which indicated that *Spondias pinnata* recorded the highest starch content (195.72 mg/100 g). The value is in line with the findings of researchers who recorded a starch content of 247 mg/100 g slightly higher than the present work while evaluating the nutritional composition in the fruits of *Spondias purpurea* [22].

As far as total free amino acid is concern, *Spondias cytherea* recorded highest total free amino acid content (158.67 mg/100 g) showing a wide variation in comparison to *Spondias axillaris* (25.33 mg/100 g) and *Spondias pinnata* (22.67 mg/100 g). The variation among the species may be due to the differences in genetic factor, soil nutrient status and environmental variations [23].

4. CONCLUSION AND FUTURE PROSPECTS

The result of the study highlighted the significance of wild underutilized fruit as a cheap source of nutrient for the rural and tribal people of the region. The fruits of *Spondias* species especially *Spondias pinnata* in particular was found to have immense sources of nutrients and should be studied further in depth to explore the hidden potential towards human health. There is a need to explore more fruit species in the wild that will add new dimensions towards nutrient composition database for the region which is

essential for health, nutrition and policy programme planning. Besides being consumed directly, focus needs to be given in secondary agriculture for developing quality value added products from these species to add value in order to enrich the underutilized fruits in the market. This will help to create income generation and improve general welfare of the community in the region.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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